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SYMPATHETIC AFFERENT IMPULSES FROM THE HEART, GREAT VESSELS, PERICARDIUM AND PLEURA OF THE CAT: THE EFFECT OF MECHANICAL AND CHEMICAL STIMULI.

by

Tone Nerdrum
cand. real., Oslo University, Norway 1974

DISSERTATION

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INTRODUCTION

Since the end of the ninteenth century the sympathetic afferent input from the cardiopulmonary region has been thought to include a system of nociceptors that signal visceral pain (White, 1957). During the past decade the idea has developed, largely on the basis of numerous studies by Brown, Malliani, Ueda, Uchida and Kampine and their colleagues (which I shall discuss in detail below), that the sympathetic afferent input from the thoracic viscera largely functions as a system of mechanoreceptors that provide tonic information about mechanical events in the heart and great vessels and are the spinal equivalent of the vagal baroreceptors and cardiac volume receptors. This theory is not altogether convincing. As we shall see, there are many aspects of sympathetic afferent innervation, such as its wide distribution in connective tissue away from major blood vessels, the existence of fibers with multiple endings, the low tonic discharge of the fibers under control conditions and their relatively small and transient response to an increase in pressure, which do not conform with the properties required in a regulating input, such as that supplied by the vagus and carotid sinus nerves. Some of these general properties were outlined in the first

electrophysiological studies in this field (Holmes and Torrance, 1959; Ueda et al., 1969), but they have been ignored in the last decade because researchers primarily selected sympathetic afferent nerve endings from particular regions (i.e. cardiovascular endings), and stressed certain aspects of their response. Although investigators have described the effects of mechanical stimuli in general terms, there has been little critical discussion of the limitations of the response of these fibers to changes in cardiovascular pressures. We know that sympathetic afferent nerve endings are stimulated by a number of chemical agents, but their role as "chemoreceptors" has been greatly overshadowed by the emphasis on their function as mechanoreceptors. Their chemical sensitivity may be of great functional importance, however, because it includes a sensitivity to substances such as bradykinin and prostaglandins that are produced in the tissues in a wide variety of circumstances.

Sympathetic afferent nerve endings are found in most thoracic organs, as well as in investing tissue like pericardium and pleura. The afferent fibers follow the cervical sympathetic cardiac nerves or direct thoracic sympathetic nerves to the stellate ganglion and sympathetic chain, where they may either ascend or descend in the chain before entering the spinal cord via the white rami. Sympathetic afferent fibers have their cell bodies in the dorsal

root ganglion, and they terminate by making synaptic contact in the dorsal horn of the spinal cord. The anatomy of the thoracic sympathetic innervation is described in Appendix I.

TERMINOLOGY

The correct terminology for visceral afferent fibers which enter the spinal cord with the sympathetic nerves is still debated. According to the conventional view presented in neuroanatomical textbooks such as Human Neuroanatomy by Carpenter (1976), the sympathetic nervous system is a purely efferent system conveying motor nerves to the heart, smooth muscle, glands and viscera. The spinal visceral afferent nerves have been omitted from the sympathetic nervous system on the grounds that they have no reflex influence on sympathetic efferent discharge, and in terms of function, generally resemble the somatic afferent fibers.

J. N. Langley, who laid the foundation of our knowledge of the anatomy and physiology of the sympathetic nervous system, is mainly responsible for defining the system as consisting of motor pathways only. Although he recognized the presence of sensory fibers in the sympathetic nerves (Langley, 1900, 1903) and even used the term "afferent sympathetic fibers" in his review, Langley (1903) felt that their only function was to convey a poorly localized sensation of pain from the viscera, and that they

were incapable of evoking reflex changes in sympathetic efferent activity. This view was based on experiments in which electrical stimulation of the cut central end of the hypogastric nerve led to contraction of various pelvic organs (Langley and Anderson, 1894). Although this effect was not seen in animals in which the splanchnic nerves had been sectioned and allowed to degenerate, Langley believed that it did not involve the central nervous system, but was due to antidromic stimulation across the ganglion of sympathetic efferent nerves to the pelvic viscera, rather than to a sympatho-sympathetic reflex arc that included a central component.

We now know that sympathetic afferent stimulation leads to reflex changes in automonic function. Malliani and coworkers showed that stimuli (such as an increase in coronary flow or pressure, experimental coronary occlusion or increased blood pressure) which excite sympathetic afferent nerve fibers evoke a reflex change in efferent sympathetic preganglionic activity to the heart in vagotomized, spinal cats (Malliani et al., 1969; Brown and Malliani, 1971; Malliani et al., 1971). The response was abolished by section of the stellate ganglion (Malliani et al., 1971). Pagani et al. (1974) found that stimulation of cardiac sympathetic afferent fibers alone always led to an increase in preganglionic efferent discharge, whereas simultaneous stimulation of cardiac and aortic afferent

fibers had variable effects. Stimulation of sympathetic afferent fibers from the heart also led to a reflex change in activity of vagal efferent fibers to the heart (Schwartz et al., 1972). Electrical stimulation of sympathetic afferent fibers or chemical stimulation of their endings (i.e. by veratridine) usually had an excitatory effect upon cardiovascular functions in vagotomized, spinal cats. It produced a reflex increase in blood pressure (Peterson and Brown, 1971; Lioy et al., 1974; Uchida, 1975b), an increase in myocardial contractility (Malliani et al., 1972) and an increase in heart rate (Malliani et al., 1973). In light of what we now know about the reflex effects of spinal afferent stimulation, therefore, it seems that the term "sympathetic afferent" is properly applied to these nerve fibers.

Although Langley acknowledged the existence of afferent fibers in the sympathetic nerves (Langley and Anderson, 1894; Langley, 1900, 1903), little was known at the time about their functional properties except that they signalled visceral pain. Already in 1899, Francois-Franck proposed that cardiac pain could be relieved by section of the sympathetic nerves from the heart. In the first few clinical studies, in which only the cervical sympathetic chain was excised bilaterally, 60 percent of the patients were relieved of cardiac pain (Jonnesco, 1920; Cutler, 1927), and the success rate improved considerably when the

procedure was later extended to include bilateral section of both the stellate ganglion and the upper four thoracic sympathetic ganglia (White et al., 1933; Lindgren and Olivecrona, 1947; White, 1957). Similarly, vascular pain produced by an aneurysm of the aortic arch or descending thoracic aorta, could be completely relieved by blocking the sympathetic afferent pathway from the area (White, 1932).

ELECTROPHYSIOLOGICAL STUDIES

The first electrophysiological study of activity in individual sympathetic afferent fibers was by Holmes and Torrance (1959), and the next investigation followed 10 years later (Ueda et al., 1969). In the last decade, however, there have been many studies of activity in sympathetic afferent fibers from the thorax. Sympathetic afferent fibers have been examined by conventional electrophysiological techniques using single fiber or small multifiber preparations; the location of the endings has been determined by punctate stimulation, and the functional characteristics of the endings have been determined by analysis of their resting discharge and their response to mechanical and chemical stimuli.

Holmes and Torrance (1959) recorded impulses from nerves entering or leaving the stellate ganglion in the

cat, and examined activity in sympathetic afferent fibers that supplied a variety of thoracic structures. Although they found nerve endings in such different structures as the longus colli muscle and the lung root, their findings suggested that many of the sympathtic afferent fibers had endings in the pleura or connective tissue investing thoracic organs, and that many fibers branched to supply multiple terminals.

Ten years later, Ueda et al. (1969) described the distribution of sympathetic afferent fibers from the thorax in dogs. Like Holmes and Torrance (1959), they found a dense distribution of endings in the upper mediastinal pleura, but they also found many endings in the walls of the heart and great vessels including the aortic arch.

Since 1969, there have been many electrophysiological studies of the sympathetic afferent innervation of the thorax in cats and dogs, and these more recent studies have provided much information about endings in a number of vascular locations. Investigators have examined sympathetic afferent endings in the ventricles (Brown and Malliani, 1971; Malliani et al., 1973; Nishi and Takenaka, 1973; Uchida et al., 1974; Hess et al., 1974; Kostreva et al., 1975; Uchida, 1975a; Nishi et al., 1977; Purtock et al., 1977), atria (Uchida and Murao, 1974c; Uchida, 1975a), pulmonary artery (Nishi et al., 1974), pulmonary veins

(Lombardi et al., 1976) and lung parenchyma (Kostreva et al., 1975), as well as endings in the aortic arch and descending aorta (Uchida, 1975b; Pagani, 1975; Malliani and Pagani, 1976). Thus, in contrast to the first two papers by Holmes and Torrance (1959) and Ueda et al. (1969), which reported an extensive sympathetic afferent innervation of the pleura and mediastinal connective tissue, research in the last decade has concentrated more on fibers with endings in the wall of the heart and great vessels. Moreover, in their intensive study of cardiovascular endings, and the role of these endings in cardiovascular regulation, later workers have tended to overlook the general principles of afferent sympathetic innervation that were so clearly outlined in earlier studies. They have also tended to make too close a comparison between the sympathetic and the vagal afferent inputs, and have ignored many of the important differences that exist between those two systems.

In this thesis I shall investigate a large sample of sympathetic afferent endings located in various structures, and by examining their response to mechanical and chemical stimuli I hope to return to a more general description of the common properties of the fibers as a whole, and to question some of the more recent theories about their primary function and their role in cardiovascular regulation.

GENERAL PROPERTIES

Although the majority (73 percent) of sympathetic afferent fibers are unmyelinated C-fibers - the remaining 27 percent being small myelinated fibers in the Aδ range (Saccomanno, 1943) - most of the information on sympathetic afferent fibers has been obtained by examining the small myelinated fibers. We know comparatively little about the properties of the unmyelinated component.

As indicated by Holmes and Torrance (1959) sympathetic afferent nerve fibers may have more than one functional ending. Most studies in the late 60's and early 70's made no reference to fibers with multiple endings. From 1973, however, the literature contains a few accounts of multi-terminal sympathetic afferent fibers (Malliani et al., 1973; Uchida and Murao, 1974c; Nishi et al., 1974; Malliani and Pagani, 1976; Nishi et al., 1977), but although the occurrence of these fibers was acknowledged, they received little attention, and were not included in discussions of the function of sympathetic afferent fibers as a whole. This is surprising since fibers with multiple terminals are a well recognized feature of the sympathetic afferent innervation of the abdominal viscera (Bessou and Perl, 1966; Morrison, 1973; Floyd and Morrison, 1974). The existence of sympathetic afferent fibers with multiple endings has not been examined in a systematic way, and in

this thesis I shall examine the incidence of fibers with multiple endings and describe their patterns of distribution.

MECHANICAL SENSITIVITY

Sympathetic afferent fibers from the thorax are described as having a low tonic discharge, most fibers firing with less than one imp/sec (Nishi et al., 1974; Malliani and Pagani, 1976). Whereas the majority of C-fibers have an irregular firing pattern independent of pressure changes in the corresponding vascular compartment (Ueda et al., 1969; Uchida and Murao, 1974c; Uchida, 1975a), most Aδ fibers fire with a cardiovascular rhythm (Ueda et al., 1969; Malliani et al., 1973; Uchida et al., 1974; Uchida and Murao, 1974c; Nishi et al., 1974; Uchida, 1975a and b; Lombardi et al., 1976). This cardiac rhythm was one of the features of the Aδ fibers that persuaded Malliani et al. (1975) that sympathetic afferent fibers have an important mechanoreceptor function, signalling changes in cardiac pressure and volume. Descriptions of Aδ fibers supplying the aorta (Malliani and Pagani, 1976) suggest, however, that an irregular pattern of discharge is not unusual, and in early experiments in this study I found many cardiovascular afferent fibers which had an irregular firing pattern under control conditions. Since their pattern of discharge is so important to any discussion of the function

of sympathetic afferent fibers, I have attempted to evaluate the resting discharge of a large group of afferent sympathetic fibers supplying a variety of cardiovascular structures.

In one respect, the mechanosensitive properties of the sympathetic afferent endings are particularly puzzling. All investigators have found the endings to be exquisitely sensitive to touch, firing with bursts of impulses to the slightest probing of their receptive fields. By contrast, the endings are, in general, remarkably insensitive to changes in transmural pressure. When intravascular pressure is increased by rapid injection of fluid, by i.v. infusion of pressor agents or by rapid occlusion of the pulmonary artery or descending aorta, sympathetic afferent fibers from the heart, great vessels or thoracic aorta respond with an increase of usually no more than one to eight impulses per heart beat (Ueda et al., 1969; Brown and Malliani, 1971; Malliani et al., 1973; Nishi et al., 1974; Uchida and Murao, 1974c; Uchida, 1975a and b; Lombardi et al., 1976; Malliani and Pagani, 1976). Moreover, the effect is quite transient, the endings apparently responding primarily to the rate of change of pressure, and their frequency of firing rapidly falls off even though pressure is maintained.

Most of the information about the mechanical sensitivity

of afferent sympathetic fibers in the heart and great vessels has been drawn from information obtained from multifiber recordings (Ueda et al., 1969; Uchida and Murao, 1974c; Hess et al., 1974; Uchida, 1975a and b; Kostreva et al., 1975; Purtock et al., 1977). In addition, investigators have often used elaborate experimental preparations involving complete cardiopulmonary bypass and inflation of balloons in the chambers of the heart, a technique unlikely to provide insight into the normal response of the endings, and which might produce adventitious stimulation of endings outside the heart (Hess et al., 1974; Kostreva et al., 1975; Purtock et al., 1977). In this study I have recorded impulses from "single" sympathetic afferent fibers with endings in the pericardium and pleura, as well as in cardiovascular structures, and I have examined the response of a large number of fibers to an increase in arterial blood pressure, produced by snaring the thoracic aorta. Although examination of the mechanosensitive properties of the sympathetic afferent endings was not the primary purpose of this thesis, it was nevertheless an important one. As a preliminary to examining the response to chemical agents known to be released by cardiovascular structures, I thought it essential to determine the response of my sample of fibers to changes in pressure in the physiological range. In particular I wished to determine whether the general characteristics of the fibers in my sample were similar to

those of the fibers described by previous investigators.

CHEMICAL SENSITIVITY

Largely from the studies of Malliani and his coworkers, sympathetic afferent fibers from the thorax have come to be considered as a more or less homogenous group of cardiovascular afferent fibers with mechanosensitive endings. These afferent fibers were also found to be sensitive to a number of chemical agents that were injected into the blood stream or dripped on to the receptor site. Sympathetic afferent fibers are now known to be excited by exogenous agents like veratridine (Brown and Malliani, 1971; Malliani et al., 1973; Takenaka et al., 1975; Nishi et al., 1977), by asphyxia (Ueda et al., 1969; Takenaka, 1970; Nishi and Takenaka, 1973; Uchida, 1975a) and by a number of endogenous agents such as adenosine and its phosphorylated derivatives (Uchida et al., 1969), KCl (Uchida and Murao, 1974b and c), lactic and pyruvic acid (Uchida et al., 1969; Uchida and Murao, 1974c; Uchida, 1975a; Uchida and Murao, 1975), acetyl choline (Uchida et al., 1969; Nishi and Takenaka, 1973; Uchida, 1975a), histamine and serotonin (Nishi et al., 1977) and by bradykinin (Uchida et al., 1969; Uchida and Murao, 1974a; Takenaka et al., 1975; Nishi et al., 1977). In addition, there are afferent sympathetic endings that are relatively insensitive to probing, but are very sensitive to chemical agents (Nishi and Takenaka, 1973;

Uchida and Murao, 1974c; Nishi et al., 1977).

Reviewing the literature on afferent sympathetic fibers one begins to suspect that the baroreceptor-like properties of these fibers have been overemphasized, and that too little attention has been paid to the effect of chemical stimulants. By investigating both the mechanical and chemical sensitivities of a large number of afferent sympatethic fibers, I hope to obtain some insight into the relative importance of the two modes of stimulation.

SYMPATHETIC AFFERENT FIBERS AND MYOCARDIAL ISCHEMIA

Sympathetic afferent fibers from the heart signal cardiac pain, and electrophysiologic studies showed that they are excited by experimental coronary occlusion (Brown, 1967; Ueda et al., 1969; Brown and Malliani, 1971). However it is not known whether the primary stimulus for sympathetic afferent fibers during coronary occlusion is due to mechanical changes in the heart, or to metabolites released by the ischemic tissue. Although Aδ fibers appear to be stimulated by experimental coronary occlusion only when the myocardial ischemia results in abnormal distension of the ventricular wall (Malliani et al., 1973; Uchida and Murao, 1974d), C-fiber endings appear to be stimulated directly by chemical agents released by the ischemic myocardium (Uchida and Murao, 1975). There is also evidence

that endings of both Aδ and C-fibers are stimulated by chemical agents after the coronary occlusion has been released, producing the so-called after-discharge (Uchida and Murao, 1975).

A number of chemical agents are released from the ischemic heart, such as adenosine and its phosphorylated intermediates (Imai et al., 1962), K^+-ions (Haddy and Scott, 1971), lactic acid (Conn et al., 1959), bradykinin (Furukawa et al., 1969; Kimura et al., 1973) and prostaglandins (Wennmalm et al., 1974; Block et al., 1975; Berger et al., 1976), and all may have a stimulant role during myocardial ischemia. But, of all these agents, bradykinin seems to be the most likely candidate for the role of mediator of cardiac pain.

Bradykinin is an extremely potent pain-producing agent present in various protein rich inflammatory exudates (Armstrong et al., 1957). Normal blood contains large amounts of its precursor kininogen, and has the potential for forming pharmacologically massive amounts of kinin. The pain-producing properties of bradykinin was first examined in the 1960's, and bradykinin was found to cause pain when applied to most tissues. It produced a burning pain of long latency when applied to a denuded blisterbase in the skin (Armstrong et al., 1957; Elliott et al., 1960), when injected intradermally or intrapertitoneally (Cormia

and Dougherty, 1960; Collier and Lee, 1963; Dickerson et al., 1965; Lim et al., 1967), or when injected intra-arterially in man or lightly anesthetized animals (Burch and DePasquale, 1962; Guzman et al., 1962; Lim et al., 1964; Sicuteri et al., 1966). Compared to other algesic agents, such as acetylcholine, serotonin and histamine, bradykinin was far more potent, and evoked pain when as little as 1-2 μg was injected into various somatic, abdominal, thoracic and cranial vascular beds (Guzman et al., 1962).

It soon became evident that pain induced by bradykinin could be reduced or abolished by previous administration of aspirin or indomethacin (Coffman, 1964; Lim et al., 1964; Sicuteri et al., 1966; Lim et al., 1967; Collier et al., 1968). Lim et al. (1964) showed that aspirin exerted its analgesic effect at the periphery, i.e. at the pain receptors, and it was at first thought to block bradykinin-receptors on the cell membrane. However, some years later Vane and his coworkers presented evidence that aspirin-like drugs inhibit the biosynthesis of prostaglandins (Piper and Vane, 1969; Vane, 1971; Smith and Willis, 1971; Ferreira et al., 1971), and this suggested that many of the effects of bradykinin were associated with activation of a prostaglandin generating system. Subsequently, bradykinin was found to release prostaglandins from a number of tissues, such as the dog kidney (McGiff et al., 1972), the dog spleen (Moncada

et al., 1972; Ferreira et al., 1973) and the isolated perfused rabbit ear (Juan and Lembeck, 1976; Lembeck et al., 1976). One explanation of the effect of aspirin on bradykinin-induced pain is that aspirin inhibits the synthesis and release of prostaglandins, which themselves are algesic agents and mediate the pain evoked by the bradykinin. The other possibility is that the prostaglandins may have no direct algesic effect by themselves, but may make the region hyperalgesic - i.e. sensitized - to mechanical or chemical stimuli. Early reports on the algesic properties of prostaglandins were somewhat contradictory. In man, PGE_1 did not cause pain when 0.1-100 µg in saline was applied to blisterbase skin (Horton, 1963), nor did 25-100 ng of prostaglandins (E_1, E_2, F_1 and F_2) injected intradermally (Crunkhorn and Willis, 1971). However, prolonged (i.e. hours) infusion of prostaglandins (5-10 µg/min) into superficial veins of the hand and forearm caused pain (Karim, 1971; Collier et al., 1972; Gillespie, 1972), and intraperitoneal injection of 0.01-1 mg/kg prostaglandins in mice induced a pain-like writhing response (Collier and Schneider, 1972). Ferreira (1972) then demonstrated that although intradermal injections of lipoperoxides and prostaglandins in µg doses caused overt pain in man, in the concentrations likely to occur in inflammatory reactions (ng/ml) their effect was that of long lasting hyperalgesia, i.e. sensitization, of the pain

receptors. This has been confirmed in a number of reflex studies in lightly anesthetized animals (Ferreira et al., 1973; Moncada, et al., 1974; Juan and Lembeck, 1974; Lembeck and Juan, 1974).

Of particular importance to my thesis is the work by Staszewska-Barczak et al. (1976), who applied bradykinin to the left ventricular surface in dogs, and evoked a nociceptive pressor reflex (increased heart rate and blood pressure) that was unaffected by bilateral vagotomy but was abolished by sympathectomy. The nociceptive response to bradykinin was diminished by infusion of indomethacin, but restored after small amounts of PGE_1 had been applied to the left ventricular surface over 20-30 minutes. Thus their study suggested that epicardial application of bradykinin produced pain by stimulating afferent sympathetic nerve endings in the heart, and that the algesic effect of bradykinin was potentiated by prostaglandins.

Although we know that bradykinin increases sympathetic afferent nerve activity, there is some uncertainty as to whether bradykinin only sensitizes certain nerve endings to mechanical stimuli, as proposed by Uchida and Murao (1974a), or whether it directly stimulates the endings independently of mechanical events, as suggested by Nishi et al. (1977). The effect of prostaglandins on sympathetic afferent endings has not been examined, nor has the mechanism of interaction

of bradykinin and prostaglandins on the endings been explored. Because of the postulated involvement of bradykinin and prostaglandins in the production of cardiac pain, I intend in this thesis to reexamine the effect of bradykinin on sympathetic afferent nerve endings, to study their response to PGE_1 and to examine the interactions of bradykinin and PGE_1.

METHODS

Experiments were performed on 80 cats (1.8-6.0 kg body weight) anesthetized with 30-40 mg/kg sodium pentobarbital (Nembutal, Abbott Laboratories), which was slowly infused into a forearm vein until the corneal reflex disappeared. Subsequent doses of anesthetic were given through a catheter in a femoral vein. During the recording of action potentials, the cats were paralyzed with 5 mg/kg gallamine triethiodide (Flaxedil, Davis and Geck). Periodically the effect of the blocking agent was allowed to wear off so that the level of anesthesia could be assessed and adjusted.

A tracheal cannula was inserted through a midline incision in the neck, and the lungs were ventilated with 50 percent O_2 in N_2 by a Harvard Small Animal Respirator (Model 670), whose expiratory outlet was placed under 2-4 cm of water. Tidal CO_2 was monitored (see below), and respiratory frequency and tidal volume were adjusted to keep end-tidal CO_2 at about 35-40 mmHg. I found by experience that a slow infusion of 10 mEq $NaHCO_3$ in a volume of 10 ml, 3-4 hours after the start of the experiment, kept the cat within normal acid/base limits. In arterial blood samples taken 7-8 hours after the cat had been anesthetized, arterial

PCO_2 varied from 38-42 mmHg, arterial pH from 7.35-7.42 and arterial HCO_3-ions from 22-25 mEq/l. Arterial PO_2 was always above 100 mmHg.

Rectal temperature was recorded with a probe (401) connected to a YSI Tele-Thermometer. Rectal temperature was maintained at 36-37 °C by placing the cat on a thermal water pad (Model K 1-3, Gorman-Rupp Industries Inc.), and by covering it with a blanket; additional heat was provided by two overhead lamps.

MAJOR SURGERY

I opened the chest on the left side so that I could record impulses from the left sympathetic chain and white rami. The skin was incised along the left second rib from sternum to vertebral column, and the incision was extended caudally to the level of ribs 8 or 9. The skin with cutaneous maximus muscle was separated from the underlying tissue, and all bleeding points were ligated. The following muscles were tied in slips and resected in order to expose ribs 1 to 8: latissimus dorsi, serratus ventralis, scalenus dorsalis and medius, and parts of pectoralis minor and the external oblique muscle. Ligatures were placed around ribs 2-8 dorsally and ventrally to occlude the intercostal vessels. The thorax was then opened by resecting ribs 2-7. Excessive bleeding from the cut rib ends was

stopped with bonewax (Ethicon).

The cat was placed on its right side and the thoracic window kept open by tying the cut ribs dorsally and ventrally to brass T-bars. To prevent the left lung from touching the electrodes during the recording of action potentials, a ligature was placed around the upper and middle left lung roots, and the two lobes were removed. This gave me an unhindered view of the thoracic sympathetic chain and rami and a wide exposure of the thoracic viscera, which was required when I came to determine the location of the nerve endings.

The pericardium was opened ventrally from the base of the pulmonary trunk to the apex of the heart, and the free edges were tied to a metal ring so as to suspend the heart in a cradle.

A recording pool was fashioned around the left sympathetic chain from the stellate ganglion to the 6th or 7th thoracic ganglion. Its ventral wall was formed by pleura carefully dissected free of the underlying tissue and retracted with fine sutures; and the dorsal wall was made up of the remaining chest wall. To prevent bleeding into the pool, I tied intercostal arteries and veins which crossed the floor of the pool. The pool was filled with mineral oil whose temperature was maintained at 37 °C by an overhead lamp. I repeatedly checked the pool temperature

during the experiment, particularly when measuring nerve conduction velocities.

RECORDED VARIABLES

A polyethylene catheter was passed into the aortic arch via the left femoral artery, and aortic blood pressure was recorded by a Statham transducer (P23Gb). I checked the position of the catheter tip after the chest was opened. Left ventricular pressure and left atrial pressure were recorded by Statham transducers (P23Gb) connected to catheters inserted via upper and middle left pulmonary veins.

Tracheal pressure was recorded from a sidearm of the tracheal cannula by a Statham transducer (P23Gb), and tidal CO_2 was monitored with an infrared CO_2-analyzer (Beckman LB-1). Gas samples were drawn through a microcatheter from a sidearm of the tracheal cannula to the pickup unit of the gas analyzer. Pressure in the pickup unit was reduced to about 1/2 atmosphere so that a rapid and accurate analysis of tidal CO_2 could be obtained from a small gas sample.

Electrodes for recording an electrocardiogram were placed beneath the skin of the right forelimb and left hindlimb, with a ground electrode in the neck. Afferent impulses were recorded from the left upper thoracic sympathetic chain and white rami (see below). After suitable amplification, the ECG and action potentials were continu-

ously displayed on a dual trace storage oscilloscope (549, Tektronix Inc.), and impulse activity was also monitored over a loudspeaker. In addition, a voltage analogue of impulse frequency was produced by a ratemeter (Frederick Haer and Co.) (see below).

RECORDING EQUIPMENT

The following variables were recorded on an 8-channel ink writing Grass Polygraph (Model 7): arterial blood pressure, left ventricular pressure, left atrial pressure, tracheal pressure, tidal CO_2, impulse frequency and time trace. Arterial blood pressure, left ventricular pressure, left atrial pressure, tracheal pressure and time trace were recorded, together with nerve action potentials and ECG, on a direct writing ultraviolet light recorder (SE Laboratories, 2100). In addition, all variables except impulse frequency and either left atrial pressure or tidal CO_2 were recorded on magnetic tape (Ampex, FR 1300).

NERVE RECORDING

The left stellate ganglion and its branches, the 2nd, 3rd, 4th and 5th sympathetic rami and the associated sympathetic chain were freed from surrounding tissues. The sheath was removed from the rami and the intervening chain. The 2nd-5th rami and the chain below the 5th rami were cut

and the portion of chain or ramus from which impulses were to be recorded was dissected on a small black, plexiglass platform placed at the bottom of the pool. Fine dissection was done with aid of a Zeiss microscope (magnification 10-40x).

A pair of bipolar silver recording electrodes, held by a micromanipulator, were brought into place above the nerve. One foot of the electrodes was grounded to the main nerve trunk by a thin cotton thread soaked in 0.9% NaCl solution. I dissected a small afferent filament from a ramus or from the main chain, and with fine watchmaker's forceps, I divided it into thin strands, which were placed in turn on the recording electrodes. The nerve strands were subdivided repeatedly until I obtained a "single-fiber" or "few-fiber" preparation whose action-potentials could be seen on the oscilloscope and heard on the loudspeaker.

Selection of Fibers

I routinely examined all afferent filaments whether silent or tonically active. I explored the thoracic viscera with a finger to determine the approximate location of both silent and active endings, and I examined the effect upon pulse activity of increased central aortic, left ventricular or left atrial pressure, obtained by tightening a snare around the descending thoracic aorta

just above the diaphragm. Fibers whose activity decreased immediately when aortic pressure was raised and increased when aortic pressure fell were assumed to be efferent preganglionic sympathetic fibers and were discarded (Downing and Siegel, 1963). Remaining fibers, i.e. those which either increased their impulse activity during elevated arterial blood pressure or showed no change in firing pattern were examined further.

I also examined a few "chemosensitive" fibers, whose endings were on the heart and did not respond readily to probing but which were readily stimulated by application of chemical agents such as bradykinin to the epicardium.

Location

I determined the approximate location of the afferent ending(s) whose impulses I had recorded by gently stroking the thoracic viscera with a finger tip or a cotton Q-tip moistened with 0.9% saline to find the sensitive point(s) from which bursts of activity could most easily be evoked. I determined the exact location of the ending and the extent of the receptive field using a fine glass probe or a von Frey's hair (bristle), applied at an angle of 45° to the visceral surface. When applied at an angle of 45° to the pan of a weighing balance, the bristle bent at a load of 0.1-0.2 g. When I gently stroked the receptive field(s)

with the bristle (taking care not to bend the bristle), the fiber responded with high frequency discharge that was in sharp contrast to the low frequency tonic activity. Because the great majority of sympathetic afferent fibers had an extremely low tonic discharge under control conditions, it was quite easy to locate their endings by probing the viscera in the living animal. In this respect, they were in marked contrast to vagal afferent fibers from the heart, great vessels and aorta, which under control conditions fire a conspicuous burst of activity with each heart beat. Because the background activity of vagal endings is so high, it is difficult to distinguish bursts evoked by the exploring probe from the naturally arising pulsatile discharge. Consequently the exact location of vagal endings in the heart and vessels can be determined by punctate stimulation only after the animal has been killed (Coleridge et al., 1957).

Ratemeter

As described on page 24, a voltage analogue of impulse frequency was produced by a ratemeter (Frederick Haer and Co.). An amplitude analyzer, whose window discriminator could be set to accept potentials of a particular amplitude, generated a voltage pulse for each impulse which appeared in the window. The signal was then led through a rate interval analyzer, which counted the number of pulses per

unit time. The ratemeter output was recorded by the Grass Polygraph. When I recorded impulses from slips containing two active fibers, I employed a second rate interval analyzer, which counted the action potentials that extended above the upper limit of the ratemeter window. I was thereby able to analyze impulse frequency of two fibers at the same time.

Conduction Velocity

I measured the conduction velocity of a fiber by stimulating it with single, electrical pulses and measuring the latency and conduction distance of the evoked action potential. Single electrical pulses of specific duration and voltage were generated by a Grass S-44 Stimulator and conveyed to a pair of bipolar silver stimulating electrodes. The generator pulse and the evoked action potential were displayed on a dual beam oscilloscope (RM 565, Tektronix) whose sweep was triggered at a fixed interval before each stimulating pulse.

The stimulating electrodes, with the cathode proximal, were placed either directly on the mechanosensitive ending or along the nervous pathway assumed to be taken by the afferent fiber. I examined the traces at fast speed on the oscilloscope to make sure that the spikes evoked by electrical stimulation were identical in amplitude and waveform

to those produced by mechanical probing of the ending. Records of 8-10 consecutive sweeps on the oscilloscope were taken with a polaroid camera. The conduction velocity of the afferent fiber was calculated according to the following formula:

$$C.V. = \frac{\text{conduction distance}}{\text{conduction time}} \quad m/sec,$$

conduction time being the interval from the beginning of the stimulus artefact to the beginning of the evoked action potential. The total conduction distance from the proximal stimulating electrode (cathode) to the proximal recording electrode was measured with wet thread or calipers. When measuring the total conduction distance, I assumed that all sympathetic afferent fibers passed through the stellate ganglion.

The elctrical pulses usually had a duration of 1 m/sec. Stimulating voltage varied with fiber diameter. Myelinated fibers were stimulated by 2.5-10 V, whereas unmyelinated fibers needed a higher voltage (>10 V) to evoke an action potential in the afferent fiber.

EXPERIMENTAL PROCEDURES

Effect of Increased Arterial Blood Pressure

I examined the response of afferent endings to an increase in blood pressure in the aorta and in the chambers of the left heart. Umbilical tape (Ethicon 10A) was passed loosely around the descending thoracic aorta just above the diaphragm, and the ends were pulled through a tube of polyethylene to form a snare. I increased central aortic blood pressure by tightening the snare, and I usually maintained the aortic occlusion for 15-30 seconds.

Chemical Stimulation of Endings

I examined the effect of bradykinin (BK) and prostaglandin E_1 (PGE_1) on sympathetic afferent nerve endings. These substances were applied topically to the site of the receptor. Both BK (Sigma Chemical Company) and PGE_1 (The Upjohn Company) were stored in powder form at -20 °C. Stock solutions of 1 mg/ml BK in 0.9% NaCl were stored at -20 °C and fresh solutions of 0.1-1.0 µg/ml BK were prepared for each experiment.

Because PGE_1 is not readily soluble in saline, the powder was initially dissolved in 0.1 ml absolute ethanol/mg PGE_1. Subsequent dilutions of this stock solution were with 0.9% NaCl, and I prepared a solution of 100 µg/ml PGE_1, which was kept frozen between experiments. Since

PGE_1 is unstable in aqueous solutions over a longer period of time, the latter solution was replaced approximately every 14 days. On the day of each experiment, solutions of PGE_1 (0.1-10 µg/ml) were prepared.

On most occasions, 0.5-1.0 ml of a solution of BK (1 µg/ml) or PGE_1 (0.1 or 10 µg/ml) at room temperature were dripped onto the receptor site from a syringe fitted with a long needle. The drug was applied slowly (over 3-4 seconds) to keep mechanical stimulation of the ending to a minimum. When the ending was accessible, a small square of filter paper (5 mm square) soaked in the appropriate drug solution was applied to the receptive field(s).

Since BK or PGE_1 were not injected into the blood stream but were applied topically to the receptor site, it was possible that the endings were stimulated either chemically by the solvent or mechanically by the application itself rather than by the chemical action of BK or PGE_1. I therefore applied the appropriate solvent (0.9% NaCl-solution or 10 ppm ethanol in 0.9% NaCl-solution) by topical drip or by filter paper to nerve endings, using the same mode of application as I had used for BK or PGE_1.

In some experiments I infused indomethacin (Merck, Sharp and Dohme Research Laboratories) i.v. to inhibit synthesis and release of prostaglandins. Indomethacin powder was stored at room temperature, and a solution

(10 mg/ml) was prepared as follows on the morning of each experiment: 100 mg indomethacin was dissolved in 3 ml distilled water and neutralized by the addition of 2 ml Na_2CO_3-solution (1.43 grams anhydrous Na_2CO_3 in 200 ml distilled water), and the solution was stirred for 10 minutes with a magnetic stirrer. I then added 5 ml 0.10 M Na/Na_2HPO_4-buffer (pH 7.00±0.02) to the solution and filtered it through a millipore filter (5.0 microns, Millipore Corporation), using a Swinny Adapter. The filtrate was a yellow solution of pH 7.4.

I assumed that endogenous production and release of PG was completely inhibited 20 minutes after i.v. infusion of indomethacin (personal communication, Dr. John McGiff, University of Tennessee). In early potentiation experiments I gave 2 mg indomethacin/kg body weight every two hours according to advice from Dr. McGiff. In later potentiation experiments I followed the suggestion of Dr. Gabor Kaley (New York Medical College) and increased the dose to 5 mg/kg body weight, and assumed that the inhibitory effect of the drug lasted throughout the rest of the experiment.

Exploration of Receptive Fields

I carefully mapped the area of the receptive field(s) by probing it with a von Frey's hair. To determine if the ending was located in the pleura or in the underlying

tissue, such as the aorta, I first made a hole in the pleura some distance away from the receptor. I then slipped a probe underneath the pleura, and because the pleura loosely covered the artery, I was able to elevate the pleura with the probe without touching the underlying artery. I then compared the effects upon impulse activity of stroking the pleura with those obtained by stroking the aorta. I then stripped away the pleura more extensively to determine whether the ending was in the pleura or the aorta. In experiments on some fibers I also compared the response evoked by applying BK to the pleural surface at the mechanosensitive site with that evoked by applying BK to the underlying aorta after the pleura had been stripped off.

In some experiments I accurately determined the location of a cardiac ending by punctate stimulation post mortem. I introduced probes into the chambers of the heart and compared the effects upon impulse activity of probing the inside and the outside of the heart. Using a dissecting microscope I then carefully removed the epicardium to see if impulse activity was abolished.

ANALYSIS OF DATA

Control firing frequency (imp/sec) of the afferent fiber was averaged over 15 seconds. Probing the endings

evoked high frequency bursts in the afferent fiber, and peak frequencies (imp/sec) of these bursts were estimated by counting impulses over an interval of 1/8 second at maximum firing.

The afferent response to BK or PGE_1 was characterized as follows: The latency (sec) was the time from beginning of application of the drug until impulse activity increased above control levels. Maximal frequency (imp/sec) of the response to BK or PGE_1 was counted over 3 seconds at the highest impulse activity, and maximal increase in frequency was obtained by substracting control frequency from maximal frequency. In the potentiation study I also described the afferent response as average increase in impulse activity (imp/sec), which was average firing frequency of the evoked response minus control frequency.

Mean arterial blood pressure (mmHg) was calculated as diastolic pressure + 1/3 of the pulse pressure.

For statistical analysis I used "Student T-test for paired data", and "Student T-test for unpaired data" (Snedecor and Cochran, 1967), and a significance level of 5 percent.

RESULTS

I recorded impulse activity from a total of 163 sympathetic afferent nerve fibers with endings located in the heart and great vessels, as well as in investing tissues, such as the pericardium and pleura. I selected both silent and tonically active fibers whose endings could be stimulated by probing, and which either increased their impulse activity or showed no change in impulse activity when I raised central aortic, left ventricular or left atrial pressure by tightening a snare around the descending thoracic aorta. In addition I examined a few "chemosensitive" fibers, all of which were located on the heart, and whose endings did not respond readily to probing, but which were stimulated by application of chemical agents, such as BK, to the surface of the heart.

I shall first describe the general properties of the afferent fibers, i.e. their resting discharge, conduction velocity, the incidence of fibers with multiple terminals and the general distribution of their endings. I do this in order to determine how far the fibers in my sample correspond with the fibers described by other investigators.

GENERAL PROPERTIES

Resting Discharge

At rest, the fibers had a low frequency of discharge, ranging from 0-4.1 imp/sec with an average of 0.66 imp/sec (SE±0.06). Control mean arterial pressure was 133.1 mmHg (SE±4.38). Control frequencies of fibers with cardiovascular endings were not significantly different from those of fibers located elsewhere. The control frequencies of the different fiber groups were as follows: ventricular fibers, 0.6 imp/sec (SE±0.14); left atrial fibers, 0.95 imp/sec (SE±0.42); fibers from the pulmonary veins, 0.95 imp/sec (SE±0.17); fibers from the pulmonary artery, 0.66 imp/sec (SE±0.24); aortic fibers, 0.73 imp/sec (SE±0.21); pericardial fibers, 0.7 imp/sec (SE±0.24); pleural fibers, 0.52 imp/sec (SE±0.11).

In earlier studies of sympathetic afferent fibers, Malliani, Nishi and Uchida and coworkers found that most tonically active Aδ fibers fired with a particular phase of the cardiac cycle during control condition (Ueda et al., 1969; Malliani, Recordati and Schwartz, 1973; Uchida et al., 1974; Uchida and Murao, 1974c; Nishi et al., 1974; Lombardi et al., 1976; Malliani and Pagani, 1976). However, I found that as many as 58/163 of the fibers in my sample (35.6%) (see Table 1) fired with a resting discharge that had no consistent relation to the cardiac cycle (Irregular pattern,

Fig. 1). Even if I ignored occasional irregularities of timing, I found that only 74/163 fibers (45.4%) had impulses that for any given fiber occurred at a particular phase of the cardiac cycle (Cardiac rhythm, Fig. 2), and even these fibers usually fired with only 15-30% of the heart beats. When differences in conduction velocity are allowed for (see below), the great majority of fibers fired in ventricular systole (56/74), whereas a few fibers fired in ventricular diastole (18/74). There was no difference in the pattern of discharge of fibers from the heart and great vessels or from the associated connected connective tissue. Only 8 fibers (4.9%) fired with one and occasionally two, impulses for each heart beat at normal arterial pressure ($\bar{X}$ = 129.3 mmHg, SE±5.31). In addition, some of the fibers that fired either irregularly or with cardiac events had a superimposed respiratory discharge with action potentials occurring in either inspiration or expiration.

I also recorded impulse activity from 31 fibers (19%) that had no resting discharge at control arterial pressure (137.5 mmHg, SE±3.67) but became active when I increased arterial pressure by snaring the descending thoracic aorta.

A striking feature of these results was that there was so little difference in firing patterns of fibers from the heart and great vessels or from the pericardium and pleura (see Table 1). Thus, the afferent input to the spinal cord

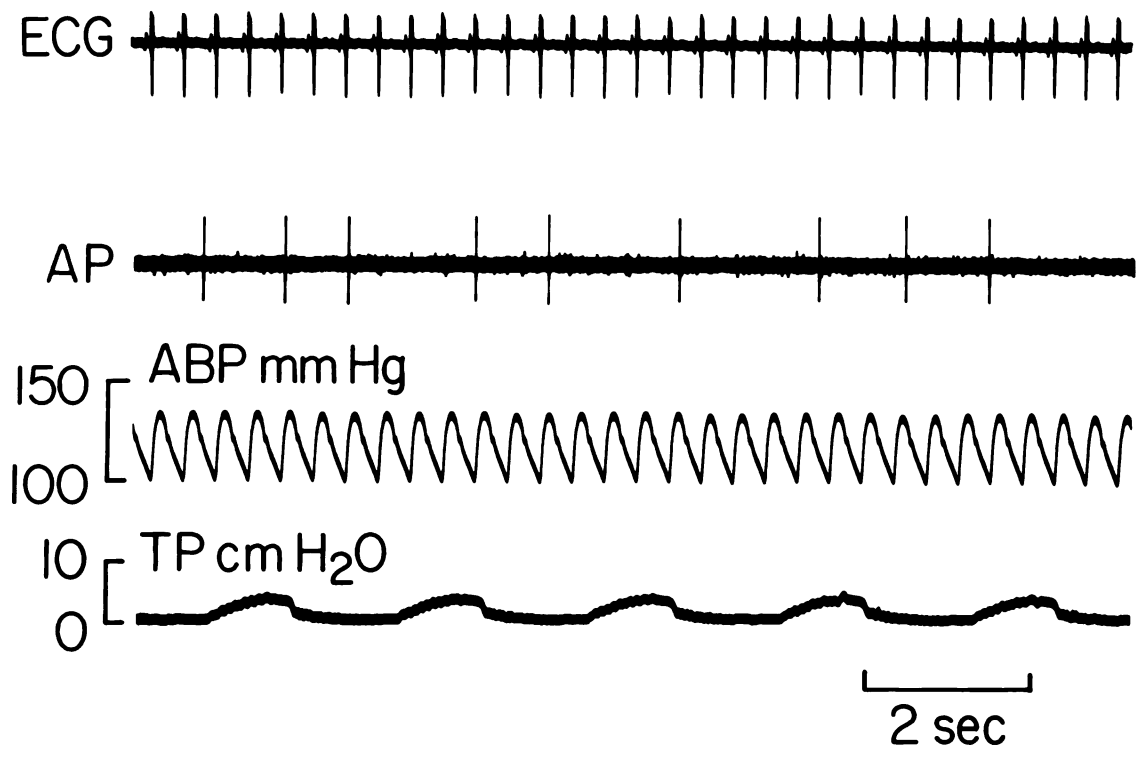

Fig. 1. Impulse activity in a sympathetic afferent fiber (conduction velocity 0.53 m/sec) with an ending in the pleura. Note that the action potentials were not consistently related to a particular phase of the cardiac cycle (Irregular firing pattern). ECG, electrocardiogram; AP, action potentials recorded from the upper left thoracic sympathetic chain; ABP, arterial blood pressure, recorded from the aortic arch; TP, tracheal pressure. Cat, open chest, positive pressure ventilation.

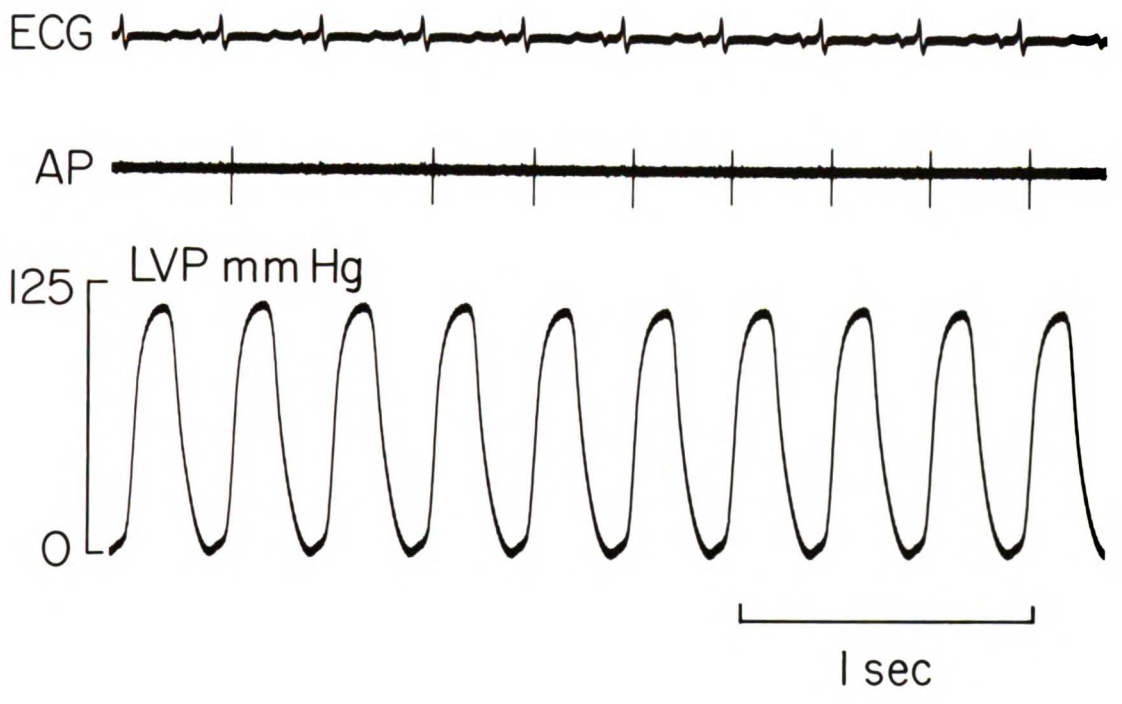

Fig. 2. Impulse activity in a sympathetic afferent fiber (conduction velocity 11.6 m/sec) with two endings on the left ventricle. Note that the action potentials always occurred in early ventricular systole (cardiac rhythm), but that not all heart beats were accompanied by an action potential. ECG, electrocardiogram; AP, action potentials recorded from the upper left thoracic sympathetic chain; LVP, left ventricular pressure.

Table 1. Incidence of firing patterns at rest in sympathetic afferent fibers with endings located in cardiovascular structures or in investing tissue.

Fiber Location	N	Irregular Pattern	Complete Cardiac Rhythm	Incomplete Cardiac Rhythm	Silent
Cardiovascular Fibers					
Cardiac fibers					
Ventricles	31	9	3	12	7
Left atrium	7	3	1	2	1
Fibers from great vessels					
Pulmonary veins	33	15	2	12	4
Pulmonary artery	10	2	0	5	3
Aorta and branches	15	5	1	9	0
Fibers from Investing Tissue					
Pericardium	14	5	1	7	1
Pleura	28	9	0	11	8
*Fibers from Aorta and Branches or Overlying Pleura	25	10	0	8	7

*In the case of aortic fibers, the pleura was removed except when marked with an asterisk.

during normal blood pressure appeared to be quantitatively and qualitatively similar for sympathetic afferent fibers from the wall of the left ventricle as from pleural fibers located a distance away from major blood vessels.

I found that the firing frequency of C-fibers at rest was similar to that of small myelinated fibers in the Aδ range (for conduction velocities; see below). Although my sample of C-fibers was small (10 fibers) it did contain each type of activity, that is, five fibers had an irregular pattern, two fibers fired with a cardiac rhythm and three fibers were silent.

In conclusion, the pattern and frequency of afferent input to the spinal cord in my sample of sympathetic afferent fibers was basically the same for all types of afferent fibers and was independent of the location of the ending, the number of endings per fiber (see below) and the diameter of the afferent fiber. This is in striking contrast to the vagal input from the various sections of the heart and great vessels.

Response to Touch

Sympathetic afferent endings in the heart and great vessels, as well as in the investing tissues, were extremely sensitive to touch. This made them very easy to locate. When I stroked the regions of the ending with a fine bristle,

afferent activity reached peak frequencies of 40-100 imp/sec, with an average of 50-60 imp/sec.

Most endings had discrete receptive fields a few mm in diameter, but occasionally the endings were sensitive to probing over a wider area of the visceral surface. One right ventricular ending, for example, was sensitive to gentle stroking of a long segment of the right ventricular wall parallel to the anterior descending coronary artery from the pulmonary conus to the apex of the heart, a total area of 40x5 mm.

Their extreme sensitivity to touch suggested that the endings were located near the outer surface of the viscera. I examined the location of several cardiac endings post mortem. Even when the endings were located in relatively thin-walled structures such as pulmonary vein or left atrium, they were more sensitive to stroking of the epicardium than of the endocardium. In the case of three left ventricular fibers, activity was immediately abolished when I pared away the epicardium.

Distribution of Endings

The 163 sympathetic afferent fibers in my sample had a total of 254 endings: 33 endings were in the left or right ventricles, 46 were at the left atriovenous junction or in the left atrium itself, 10 were in the pulmonary artery,

88 were either in the wall of the thoracic aorta and branches or in the overlying pleura, 27 were in the pericardial sac and 50 endings were in the pleura away from aorta and branches. Thus, some of the fibers had multiple endings. I shall first describe the distribution of all the endings within the heart, great vessels, pericardium and pleura, and shall then proceed to describe in more detail the fibers with multiple endings.

Of the 33 endings located in the cardiac ventricles, eight were in the right and 25 were in the left ventricle. Nishi et al. (1977) described an uneven distribution of endings in the left ventricle, with endings concentrated along the coronary arteries. I found that the endings were distributed more uniformly and were widespread in the ventricles (see Fig. 3), so that although some endings were located very close to major coronary arteries, others were located some distance away.

Of 46 endings in the left atriovenous region, only eight were in the main body of the left atrium itself. In contrast, the majority of the endings (38) were in the junction of the atrium and the pulmonary veins, a distribution resembling that of afferent vagal afferent endings in this region (Coleridge et al., 1957).

Ten fibers had endings on the pulmonary artery: seven on the left pulmonary artery, inside the pericardial sac,

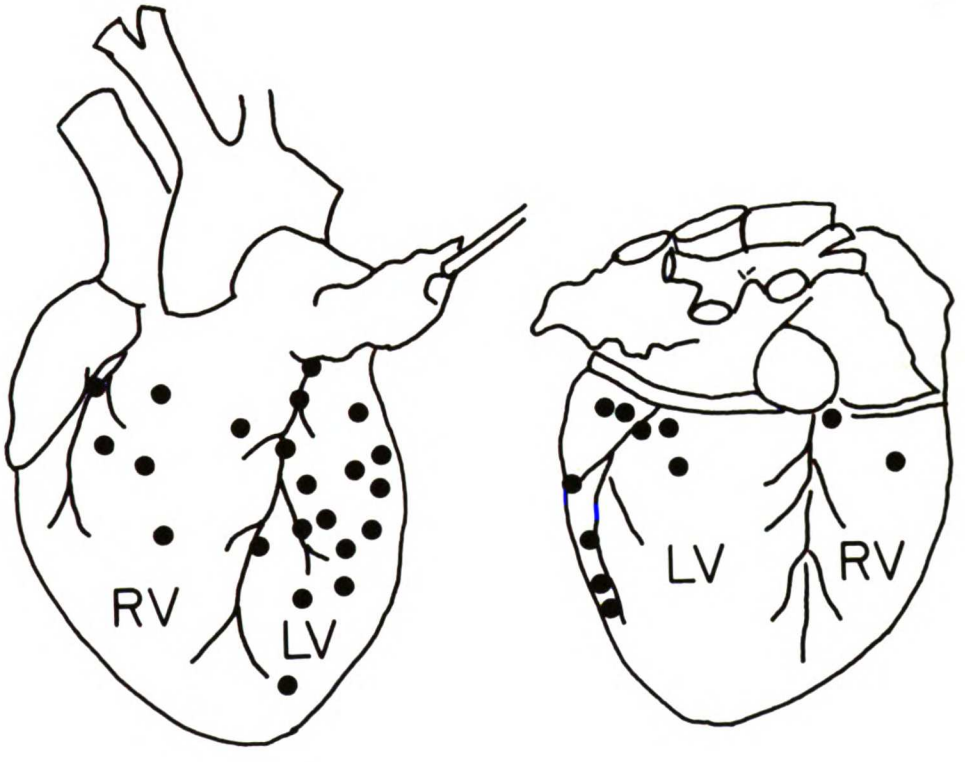

Fig. 3. Distribution of 33 sympathetic afferent nerve endings with discrete receptive fields on the right (N = 8 endings) or left ventricle (N = 25 endings). RV, right ventricle; LV, left ventricle.

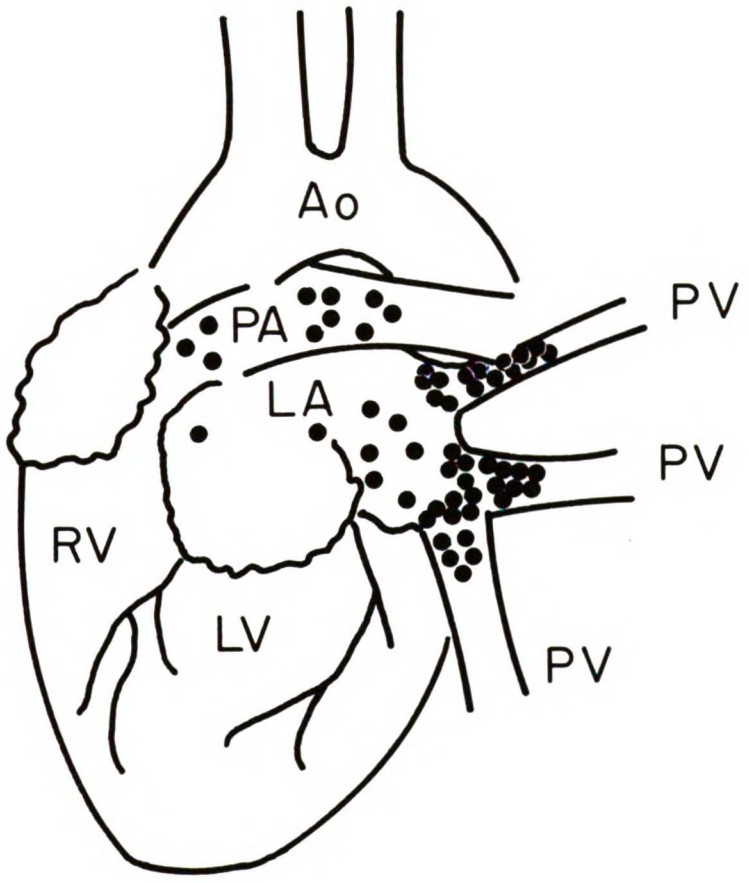

Fig. 4. Distribution of sympathetic afferent nerve endings inside the pericardium on the pulmonary trunk and left pulmonary artery, left atrium and left pulmonary veins. Ao, aortic arch; PA, pulmonary artery; LA, left atrium;PV, pulmonary veins; RV, right ventricle; LV, left ventricle.

while three endings were on the main pulmonary artery (Fig. 4).

I found a total of 88 endings that were either located in the walls of the thoracic aorta and its branches or in the overlying pleura. In the case of 13 endings, impulse activity was immediately abolished when I stripped off the pleura covering the receptor site (see Methods, p. 33), which suggests that the endings were located in the pleura overlying the arteries. The exact location of these endings is shown with other pleural endings in Fig. 7. Twenty-one endings were in the wall of the arteries themselves (Fig. 5, filled circles). These endings were still sensitive to probing after the pleura had been stripped off the receptive site. In addition, 54 endings (Fig. 7, open circles), responded with high frequency bursts when I probed specific spots on aorta and branches, but I did not remove the pleura to determine if they were located in the vessel wall or in the overlying pleura.

I found a total of 27 sympathetic afferent nerve endings in the wall of the pericardial sac. Most were located close to the left side attachment of the pericardium where their distribution appeared to follow the phrenic nerve. I only found one afferent ending on the right side of the sac (Fig. 6), but since I routinely opened the pericardial sac from the base of the pulmonary artery to

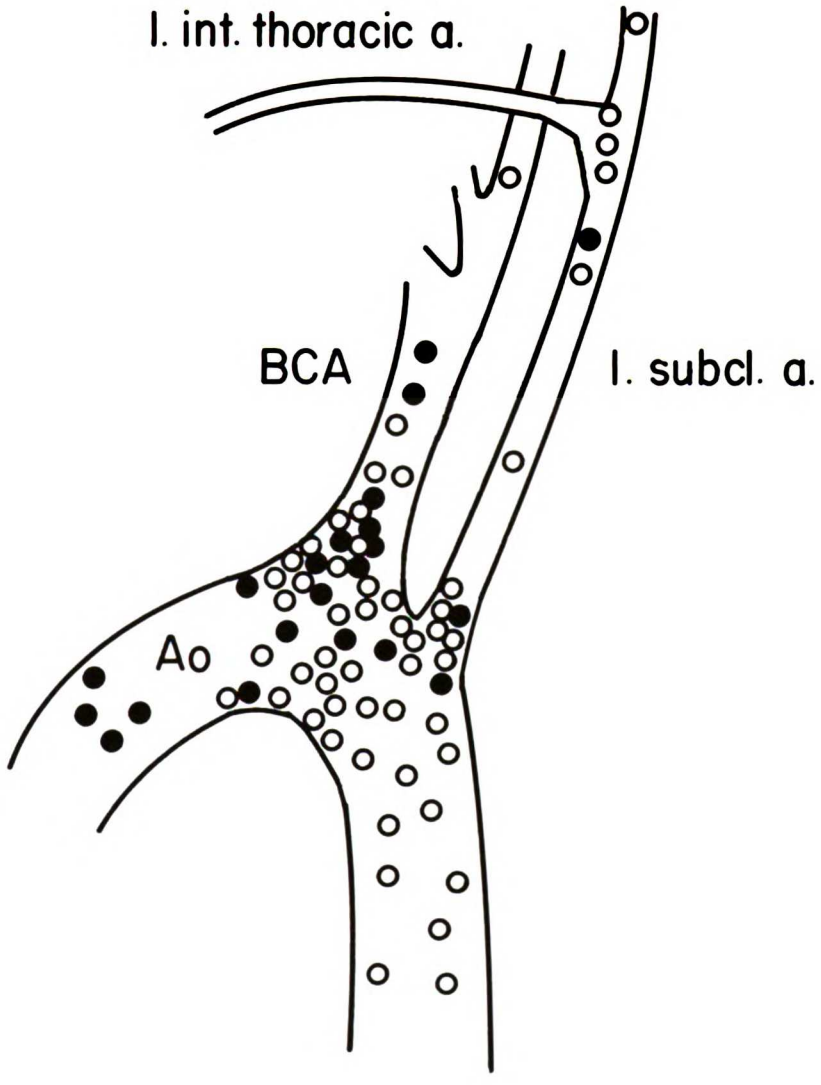

Fig. 5. Distribution of sympathetic afferent nerve endings along the thoracic aorta, the brachiocephalic artery and the left subclavian artery. ● - The pleura overlying these endings was removed to ascertain that the endings were located in the arterial wall. o - These nerve endings were located in the vessel wall or in the overlying pleura. Ao, thoracic aorta; BCA, brachiocephalic artery; l. subcl. a., left subclavian artery; l. int. thoracic a., left internal thoracic artery.

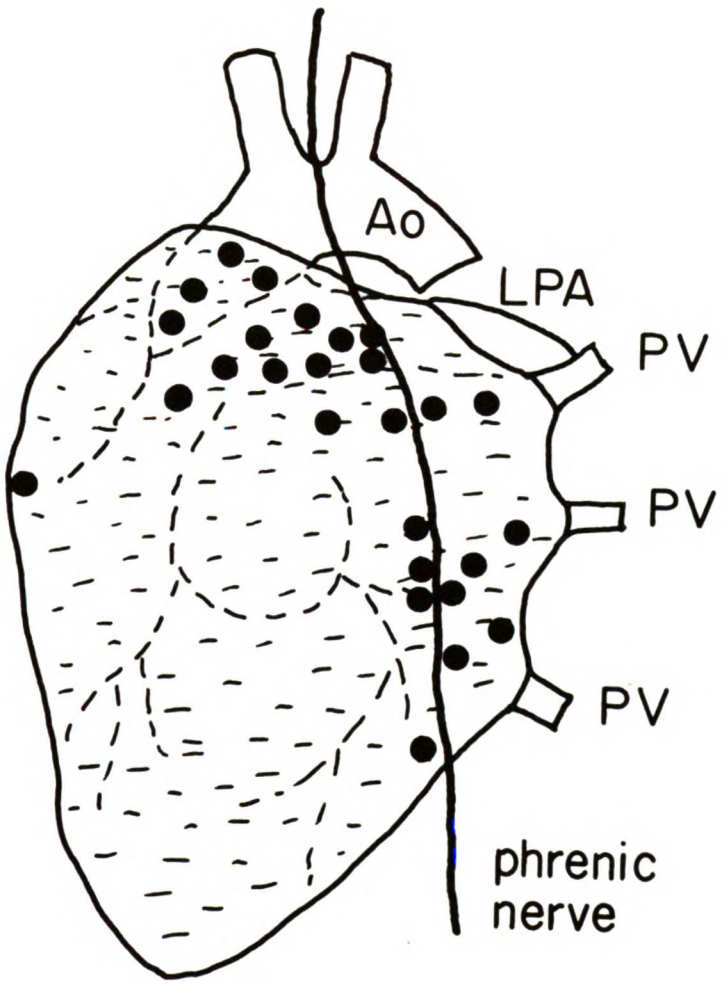

Fig. 6. Distribution of sympathetic afferent nerve endings in the pericardial sac. Ao, thoracic aorta; LPA, left pulmonary artery; PV, pulmonary veins.

the apex of the left ventricle, it is possible that I severed any left sympathetic afferent fibers that had their endings on the right side of the pericardial sac.

I also found a total of 63 sympathetic afferent nerve endings in the pleura on the left side of the thorax. Thirteen of these endings were in the pleura overlying the aorta and branches, and have been described above. When I explored the location of the pleural endings, I first made a hole in the pleura some distance away from the receptor. I then slipped a probe underneath the pleura, and because the pleura so loosely covered the viscera, I was able to elevate the pleura with the probe without touching the underlying viscera. I then compared the effects upon impulse activity of stroking the pleura with those obtained by stroking the underlying tissue, and finally I stripped away the pleura more extensively to determine whether the ending was in the pleura or in the underlying tissue.

The endings were particularly numerous in certain parts of the thoracic pleura: in the pleura overlying major arteries, distributed along the vagal and sympathetic cardiac nerves, and in the pleura at the lung roots (Fig. 7).

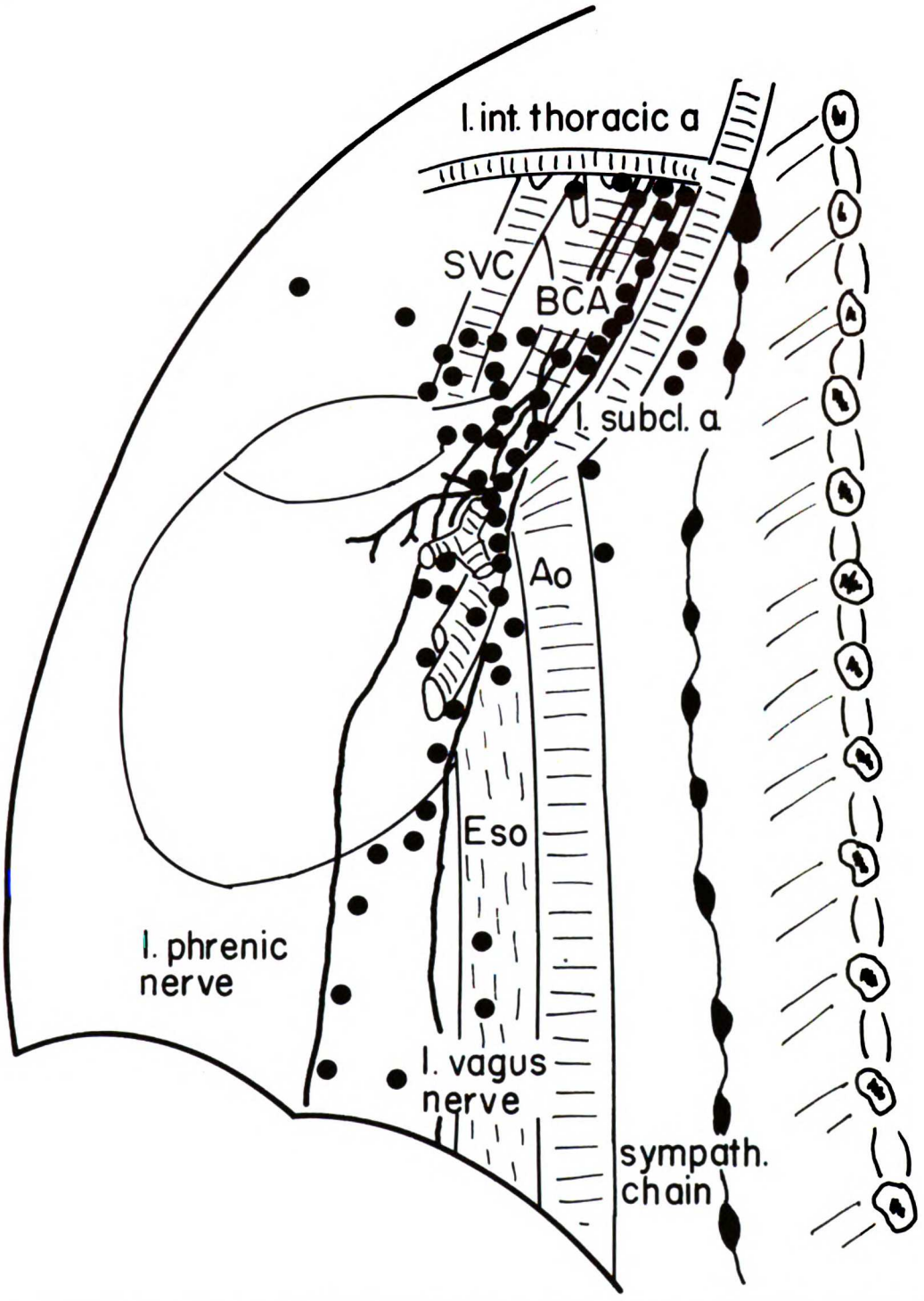

Fig. 7. Distribution of sympathetic afferent nerve endings in the thoracic pleura. l. int. thoracic a., left internal thoracic artery; SVC, superior vena cava; BCA, brachiocephalic artery; l. subcl. a., left subclavian artery; Ao, thoracic aorta; Eso, esophagus. Left lung has been removed.

Incidence of Afferent Fibers with Multiple Endings

Many fibers had 2-6 discrete, sometimes widely separated sensitive spots from which bursts of impulses could be evoked in the parent axon. Touching each of the sensitive spots of a single parent fiber with a fine bristle evoked action potentials having the identical amplitude and configuration, indicating that the sensitive spots indeed represented endings of a single branching axon of wide distribution. If two spots were touched simultaneously, there was no summation of potentials - an artefact that might be expected to occur if the activity of two axons, discharging at relatively high frequencies, was recorded simultaneously.

Of my total fiber population, 46 fibers had multiple endings, but I think that the actual incidence of multiterminal sympathetic afferent fibers may have been higher. In the first half of the study I found very few fibers with multiple endings, probably because I still lacked experience in locating the endings. However, as I became more confident in locating the endings, the proportion of fibers with multiple endings increased, so that in the final 27 experiments, 43.2% of fibers had more than one terminal, and in the final 15 experiments, 44.9% of fibers had multiple endings.

Fibers with multiple endings and those with single

endings had similar control firing frequencies (0.51 imp/sec ±0.16 and 0.58 imp/sec ±0.14, respectively).

Twenty-eight multiterminal afferent fibers had all their endings distributed either to the pleura covering the thoracic organs (16 fibers), to the thoracic aorta or overlying pleura (10 fibers), or to the heart (two fibers). Fibers supplying the pleura and pericardium often had endings distributed along small blood vessels (see Fig. 8), as described for multiterminal afferent fibers in the gut (Bessou and Perl, 1966). The remaining 18 fibers had endings distributed over a wider area of the thoracic viscera. Nine fibers, whose distribution is shown in Fig. 9, had one ending located on the heart or great vessles inside the pericardial sac, and another ending on the aortic arch. The fiber with the widest distribution (Fiber 8 in Fig. 9) had a total of six mechanosensitive endings distributed as follows: in the pleura by the left internal thoracic artery, in the pleura by rib 2, in the connective tissue between the aortic arch and the pulmonary artery, in the wall of the aortic arch, in the wall of the lower left pulmonary vein, and in the left ventricular wall.

Conduction Velocity

I measured the conduction velocities of 77 sympathetic afferent nerve fibers. The great majority (67/77) were

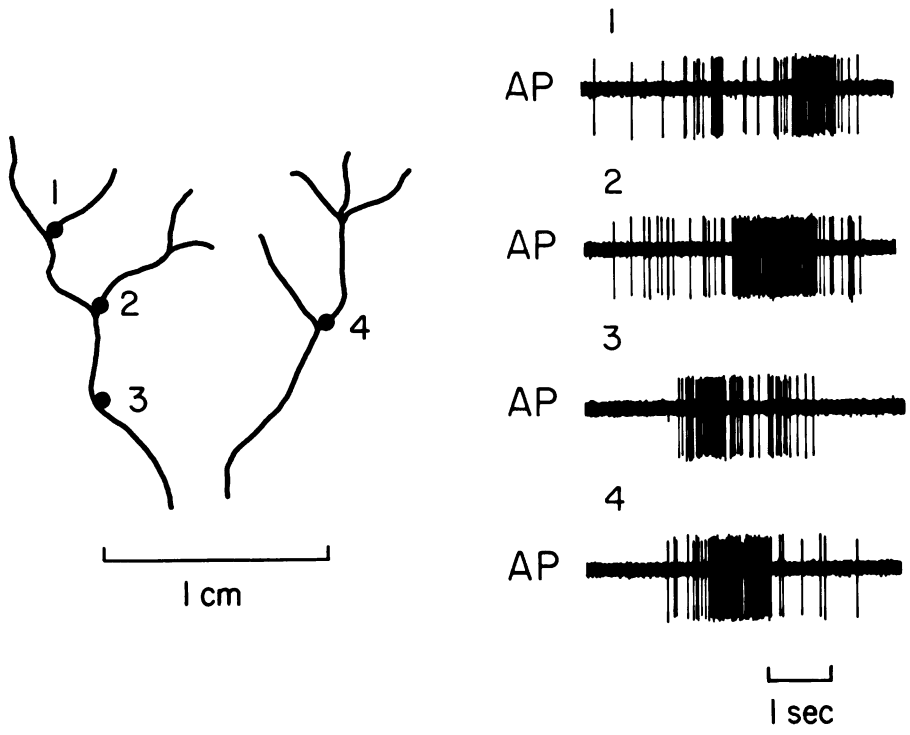

Fig. 8. On left, distribution of endings of a multiterminal sympathetic afferent fiber with conduction velocity 12.4 m/sec. The fiber had four endings 3-9 mm apart, located along small blood vessels in the pericardium. On right, high frequency bursts evoked in the afferent fiber by touching each ending with a Frey's hair. No activity could be evoked by touching the region between endings. AP, action potentials recorded from the upper left thoracic sympathetic chain.

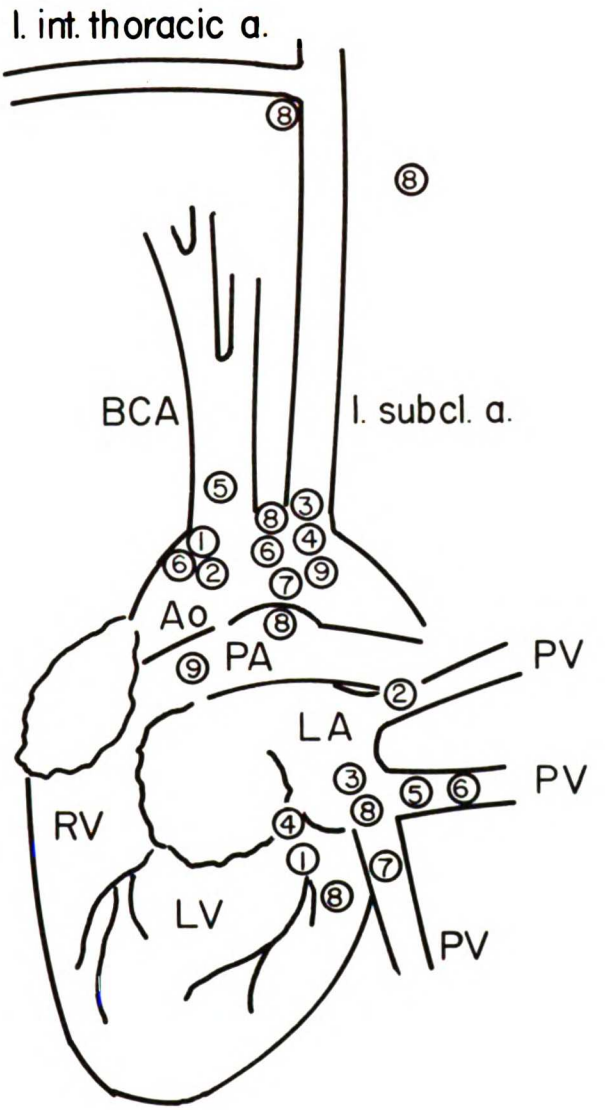

Fig. 9. Distribution of endings of 9 multiterminal sympathetic afferent fibers that had endings both on the heart, pulmonary veins or pulmonary artery inside the pericardial sac and also on the aortic arch or initial part of the brachiocephalic artery. The fibers are numbered from 1-9, and the endings of the same afferent fiber are indicated by the same number. l. int. thoracic a., left internal thoracic artery; l. subcl. a., left subclavian artery; BCA, brachiocephalic artery; Ao, thoracic aorta; PA, pulmonary artery; LA, left atrium; RV, right ventricle; LV, left ventricle; PV, pulmonary veins.

small myelinated Aδ fibers, with conduction velocities of 2.9-27.5 m/sec ($\bar{X}$ = 9.51 m/sec, SE±0.59), and only 10 of the fibers were unmyelinated C-fibers with conduction velocities of less than 2.5 m/sec ($\bar{X}$ = 1.87 m/sec, SE±0.59).

Site of Recording

I recorded impulse activity from the left sympathetic chain between the stellate ganglion and the sixth thoracic ganglion. I always recorded first from the chain below T-4 or T-5 and worked systematically through the various sections of the chain and white rami towards the stellate ganglion. I encountered most of the cardiac fibers in the chain between T-3 and T-4, whereas the fibers whose endings were located outside the heart were found equally in the chain between T-4 and T-5 and between T-3 and T-4 (Table 2).

I had less success in isolating single units from the rami themselves (see Table 2), partly because the rami were so short and because the texture of the rami made them difficult to subdivide.

Table 2. Proportion of sympathetic afferent fibers with endings in cardiovascular structures or investing fascia that were recorded from the different segments of the left sympathetic chain or white rami from T2 to T5.

Fiber Location	N	Chain T2-T3	Chain T3-T4	Chain T4-T5	Chain T5-T6	2nd Ramus	3rd Ramus	4th Ramus	5th Ramus
Cardiovascular Fibers									
Cardiac fibers									
Ventricles	31	3	21	1	1	1	2	2	0
Left atrium	7	0	7	0	0	0	0	0	0
Fibers from great vessels									
Pulmonary veins	33	2	16	11	0	0	2	2	0
Pulmonary artery	10	1	4	2	1	0	2	0	0
Aorta and branches	15	2	7	5	0	1	0	0	0
Fibers from Investing Fascia									
Pericardium	14	0	5	5	3	0	0	0	1
Pleura	28	1	8	12	6	0	1	0	0
*Fibers from Aorta and Branches or Overlying Pleura	25	3	10	9	1	0	0	1	1

*In the case of the aortic fibers, the pleura was removed except when marked with an asterisk.

RESPONSE TO INCREASED PRESSURE

Results of earlier studies by Malliani and coworkers (Malliani et al., 1973; Malliani and Pagani, 1976; Pagani et al., 1976), by Nishi et al. (1974) and by Uchida et al. (1974) suggest that sympathetic afferent fibers signal changes in pressure and volume in the various segments of the heart and great vessels. Thus, these investigators reported that when pressure was increased by i.v. infusion of pressor agents or by occluding the thoracic aorta or main pulmonary artery, sympathetic afferent fibers from the heart and great vessels gave an initial response of usually no more than 1-8 imp/heart beat, and they adapted when the pressure was maintained at a high level. In spite of the transient nature of the response, previous investigators concluded that sympathetic afferent fibers from the heart and great vessels participate in the tonic reflex control of the circulation. In order to assess the mechanosensitive properties of the sympathetic afferent endings in my sample, as a preliminary to examining their response to chemical agents, I have examined the effect of increasing the appropriate cardiovascular pressure on the activity of 38 sympathetic afferent fibers supplying the aorta and branches, the left ventricle and the pulmonary veins. Although my results are generally similar to those obtained by previous investigators, my conclusions about the role of sympathetic afferent fibers in the control of the

circulation are different.

When I occluded the descending thoracic aorta, arterial blood pressure, systolic left ventricular pressure, left ventricular end - diastolic pressure and left atrial pressure increased and reached a maximal value within 3-8 seconds. I usually maintained the occlusion for 30 seconds, but buffer reflexes caused pressure to decline from their peak values towards the end of the occlusion period.

Even in the most sensitive fibers with endings in the thoracic aorta (see below), maximum firing rate during increased pressure was only 5-7 impulses per cardiac cycle (or 15-20 imp/sec), and in the majority of fibers the response was very much less, a marked contrast to the much higher firing rates obtained by probing the ending with a fine bristle (40-100 imp/sec).

I examined the effect of increased pressure on the activity of 10 fibers with endings in the walls of aorta and branches. In each case I stripped away the pleura covering the mechanosensitive area to determine that the ending was located in the vessel wall. These fibers were therefore similar to the aortic fibers described by Malliani and Pagani (1976), and had a similar response to increased blood pressure. When I increased mean arterial pressure from 133.1 mmHg (SE±4.38) to 210.6 mmHg (SE±4.84)

by occluding the descending thoracic aorta, the fibers responded with an initial pulsatile discharge of 2-7 impulses per cardiac cycle, with a peak frequency of 7.1 imp/sec (SE±1.13). However, a striking feature of their response was that in spite of a maintained high level of pressure, impulse activity quickly declined to one impulse per cardiac cycle or less (Fig. 10). Fig. 11 shows an example of a change in impulse of an individual fiber, which fired with bursts of 2-5 impulses per cardiac cycle as arterial blood pressure increased. Fig. 12, in which impulse activity has been counted for each cardiac cycle, shows that maximal impulse activity occurred several cycles before blood pressure reached its peak. The decrease in impulse activity during aortic occlusion was not due to a decrease in arterial pulse pressure. The latter increased on average from 25.5 mmHg (SE±2.05) to 65.5 mmHg (SE±3.96) during aortic occlusion, and whereas the firing reached a maximum of 7.1 imp/sec after only two seconds of occlusion, average impulse activity had already declined to 5.2 imp/sec by the time (five seconds) arterial pulse pressure reached its maximal value. It seems likely, therefore, that sympathetic afferent fibers from the aorta and branches primarily respond to the rate of change of pressure when pressure is increasing, and that impulse activity quickly declines again when pressure is maintained.

I also examined the effect of increased aortic

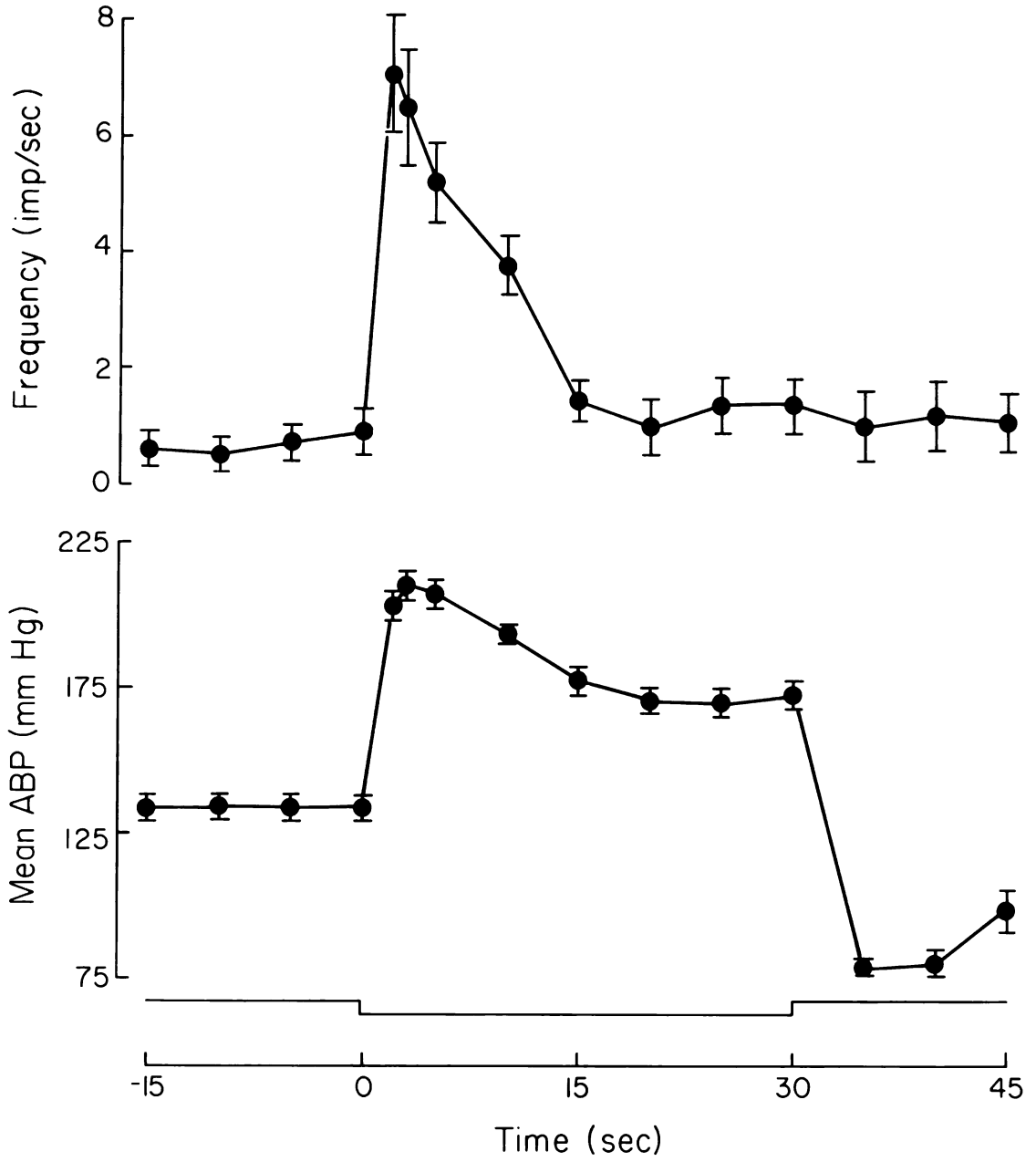

Fig. 10. Average stimulation of 10 sympathetic afferent nerve fibers from aorta and branches by an increase in arterial pressure, produced by tightening a snare around the descending thoracic aorta. The occlusion time (30 sec) is illustrated by the signal. The pleura overlying the receptive fields has been removed. Frequency, average impulse activity of the fibers ±SE; mean ABP, average mean arterial blood pressure for the corresponding occlusions ±SE. Arterial blood pressure was recorded from the aortic arch. Note adaptation in impulse activity to maintained pressure.

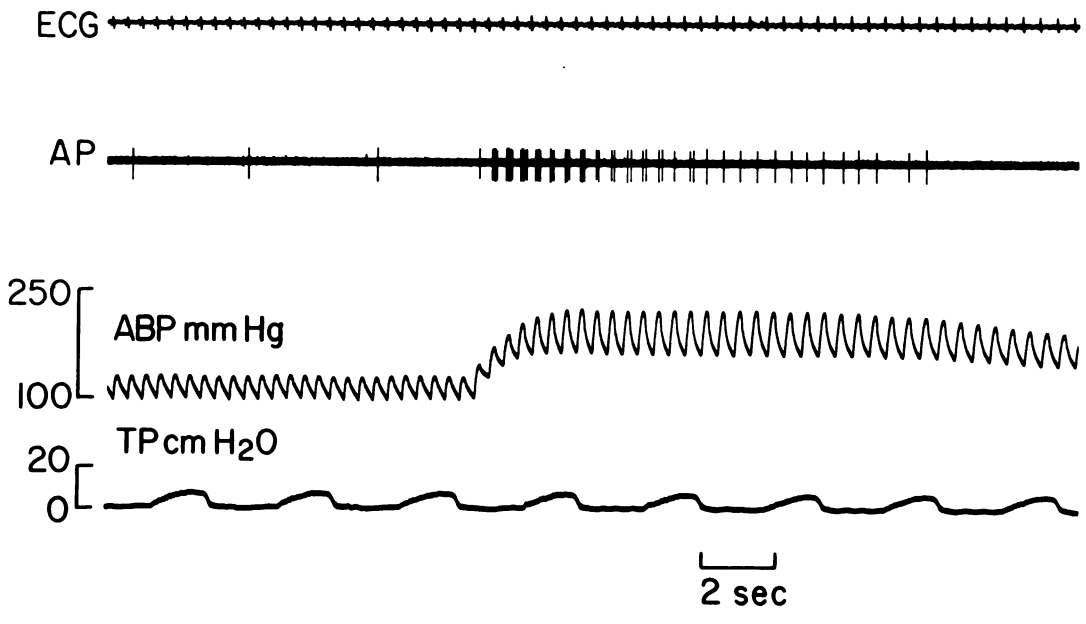

Fig. 11. Stimulation of a sympathetic afferent fiber from the brachiocephalic artery by an increase in arterial pressure, produced by tightening a snare around the descending thoracic aorta for 30 sec. The pleura overlying the receptive field has been removed. AP, action potentials, recorded from the left upper thoracic sympathetic chain; ABP, arterial blood pressure, recorded from the aortic arch; TP, tracheal pressure, positive pressure ventilation.

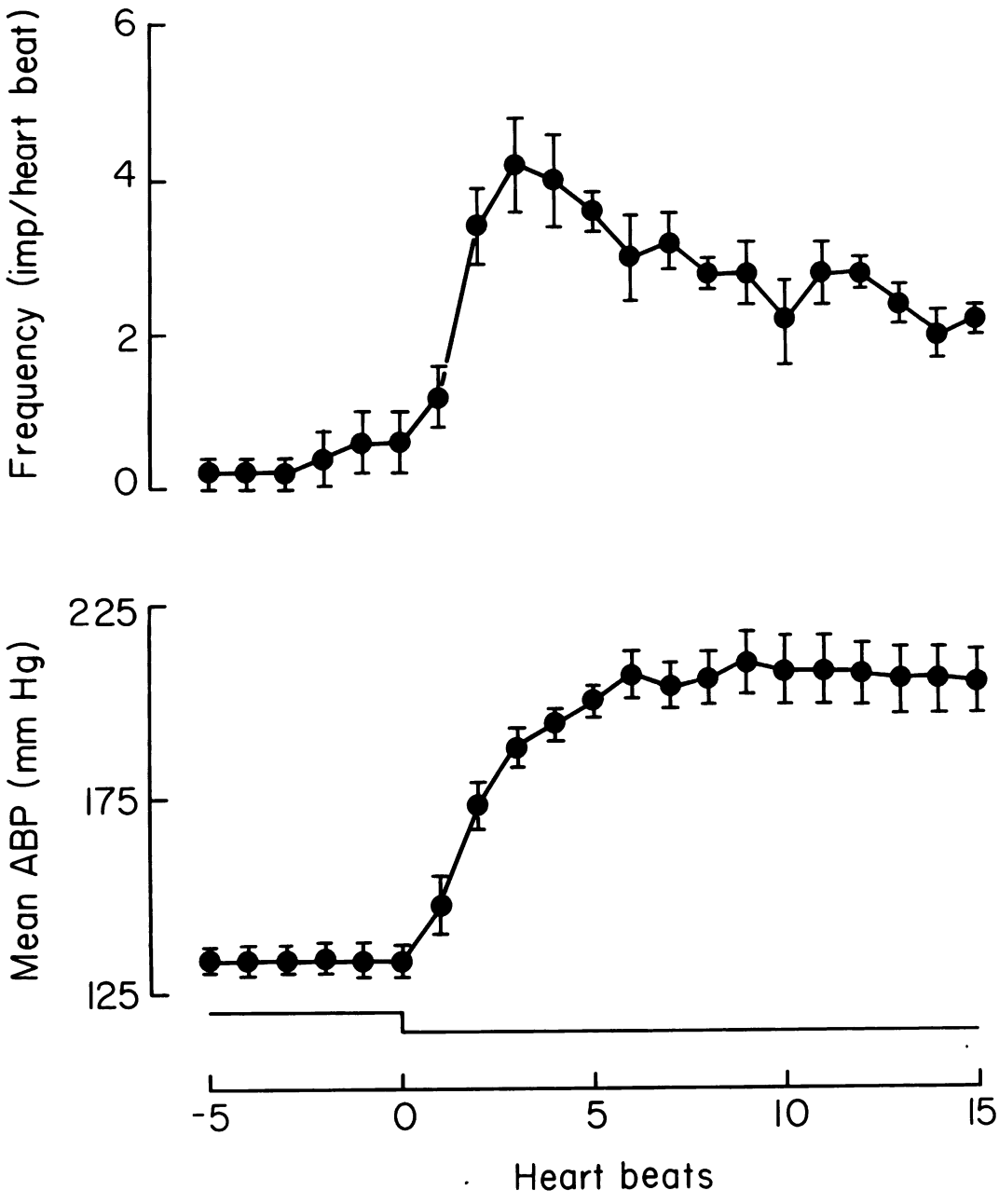

Fig. 12. Average stimulation of five sympathetic afferent nerve fibers from aorta and branches by an increase in arterial pressure, produced by tightening a snare around the descending thoracic aorta. The occlusion is illustrated by the signal. The pleura overlying the receptive fields has been removed. Frequency, average impulse activity per heart beat ±SE; mean ABP, average mean arterial blood pressure for the corresponding occlusion ±SE. Arterial blood pressure was recorded from the aortic arch.

pressure on the impulse activity of eight fibers that appeared to supply endings to the aorta and its branches, although I did not remove the pleura to determine if the endings were actually located in the vessel wall or in the overlying pleura. These fibers were therefore similar to the "aortic fibers" investigated by Uchida (1975b). For a given increase in arterial blood pressure, this group of fibers gave a qualitatively similar response to that of fibers whose vascular location was confirmed: When mean arterial blood pressure was increased from 137.9 mmHg (SE±7.98) to 215.1 mmHg (SE±10.7), the fibers gave an initial pulsatile discharge of 1-4 impulses per cardiac cycle, which quickly declined to one impulse per cardiac cycle or less. Their maximal response was smaller than that of endings whose vascular location was confirmed (5.8 imp/sec, SE±1.8), but not significantly so.

In addition, I examined the effect of aortic snare on the impulse activity of six sympathetic afferent fibers whose endings were in the pleura overlying the aorta and its branches. In all of these fibers, impulse activity was eliminated by stripping off the pleura covering the receptor site. When mean arterial blood pressure was increased from 130.5 mmHg (SE±7.14) to 206.5 mmHg (SE±3.48), the fibers fired with a maximal frequency of 5.5 imp/sec (SE±1.96). Whereas some fibers fired with only one impulse per heart beat, others responded with a pulsatile discharge

of 2-4 impulses per beat, and in all cases, impulse activity quickly declined in spite of a maintained pulsatile pressure stimulus. In conclusion, although my results suggest that fibers from the wall of the thoracic aorta are more sensitive than endings in the overlying pleura to a given increase in pressure, this is not statistically significant.

I examined the effect of increased pressure on the impulse activity of eight sympathetic afferent fibers with endings in the wall of the left ventricle. When I snared the thoracic aorta, systolic left ventricular pressure increased from 145.1 mmHg (SE±7.6) to an average peak value of 234.6 mmHg (SE±7.97), and left ventricular diastolic pressure increased on average by 5 mmHg. There has been much speculation about the proper stimulus for vagal afferent fibers from the ventricles, whether they are primarily stimulated by systolic left ventricular pressure, or whether the proper stimulus is left ventricular end diastolic pressure (Coleridge et al., 1975b). However, in this thesis I shall not get involved in the discussion about the proper stimulus for sympathetic afferent fibers from the ventricles.

Fibers with endings in the left ventricular wall gave a remarkably different response to a given increase in systolic pressure than fibers with endings in the thoracic

aorta. Although Malliani and coworkers have examined the effect of increased blood pressure on both left ventricular endings (Malliani, Recordati and Schwartz, 1973) and endings from the thoracic aorta (Malliani and Pagani, 1976), they make no reference to these differences. I found that compared to endings in the thoracic aorta, endings in the left ventricle were remarkably insensitive to a given increase in systolic pressure (Fig. 13). Changes in activity were small, and even with an increase in systolic left ventricular pressure of 70-100 mmHg, their maximal response rarely exceeded one impulse per cardiac cycle (3-4 imp/sec). Also, in contrast to fibers from the thoracic aorta (see p. 59) they maintained the increase in discharge throughout the occlusion period, showing no sign of adaptation. Typically the fibers fired with one, and only occasionally two, impulses per heart beat from the beginning of snare until the snare was released (Fig. 14), although two fibers failed to fire with each heart beat even though ventricular pressure was high (Fig. 15). Fig. 15 also shows the contrast between the response to pressure and the high frequency discharge obtained by merely touching the ending.

I also examined the response to increased pressure of six sympathetic afferent fibers with endings on the pulmonary veins. During aortic occlusion, pulmonary venous pressure increased on average by 5-6 mmHg, and the fibers

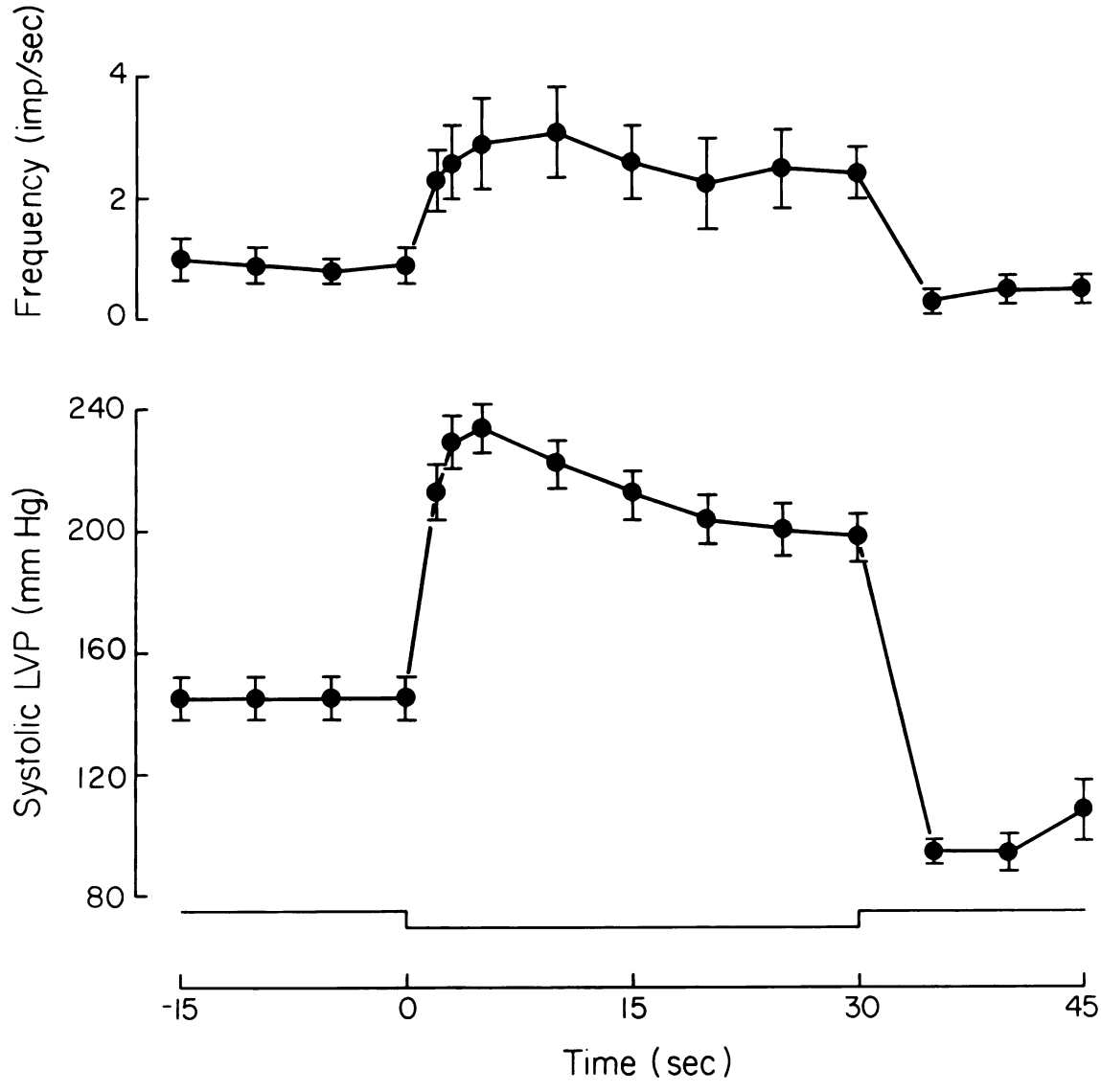

Fig. 13. Average stimulation of eight left ventricular fibers by an increase in systolic left ventricular pressure, produced by tightening a snare around the descending thoracic aorta. The occlusion time (30 seconds) is illustrated by the signal. Frequency, average impulse activity of the fibers ±SE; systolic LVP, average systolic left ventricular pressure for the corresponding occlusions, ±SE. Note that the average response was small, but that it was well sustained throuout the period of increased pressure.

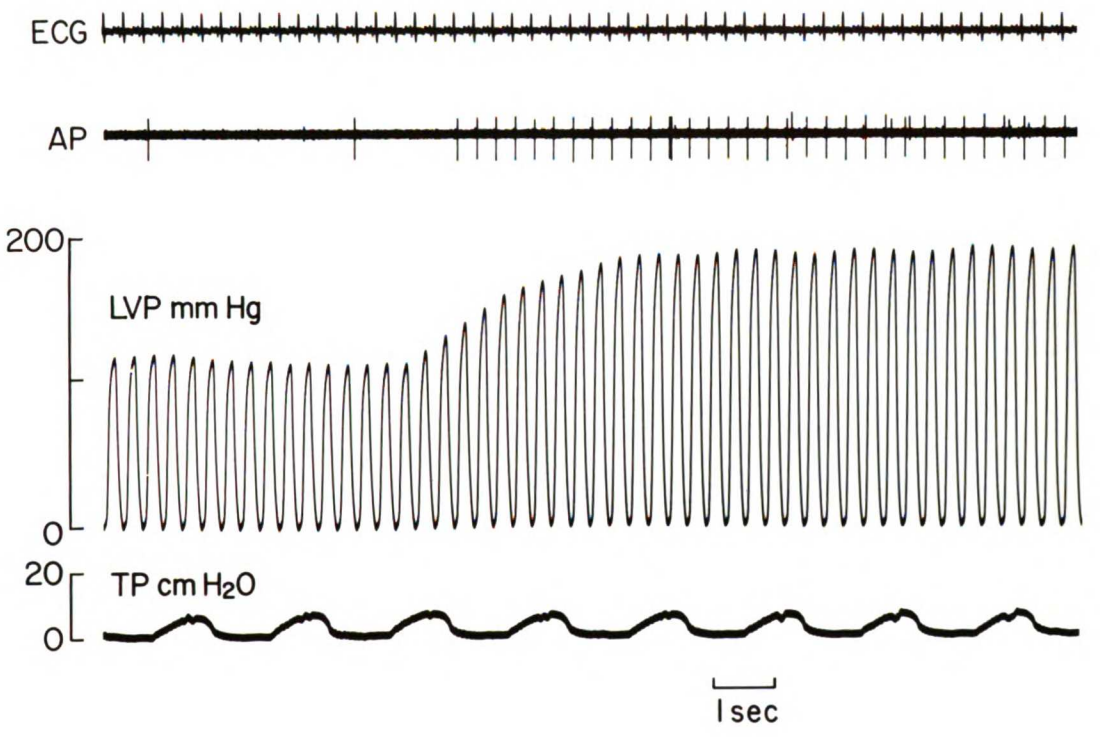

Fig. 14. Stimulation of a sympathetic afferent fiber from the left ventricle by an increase in left ventricular pressure, produced by tightening a snare around the descending thoracic aorta. Occlusion time was 30 seconds; the fiber maintained this pattern of discharge until the snare was released. AP, action potentials recorded from the upper left thoracic sympathetic chain; LVP, left ventricular pressure; TP, tracheal pressure, positive pressure ventilation.

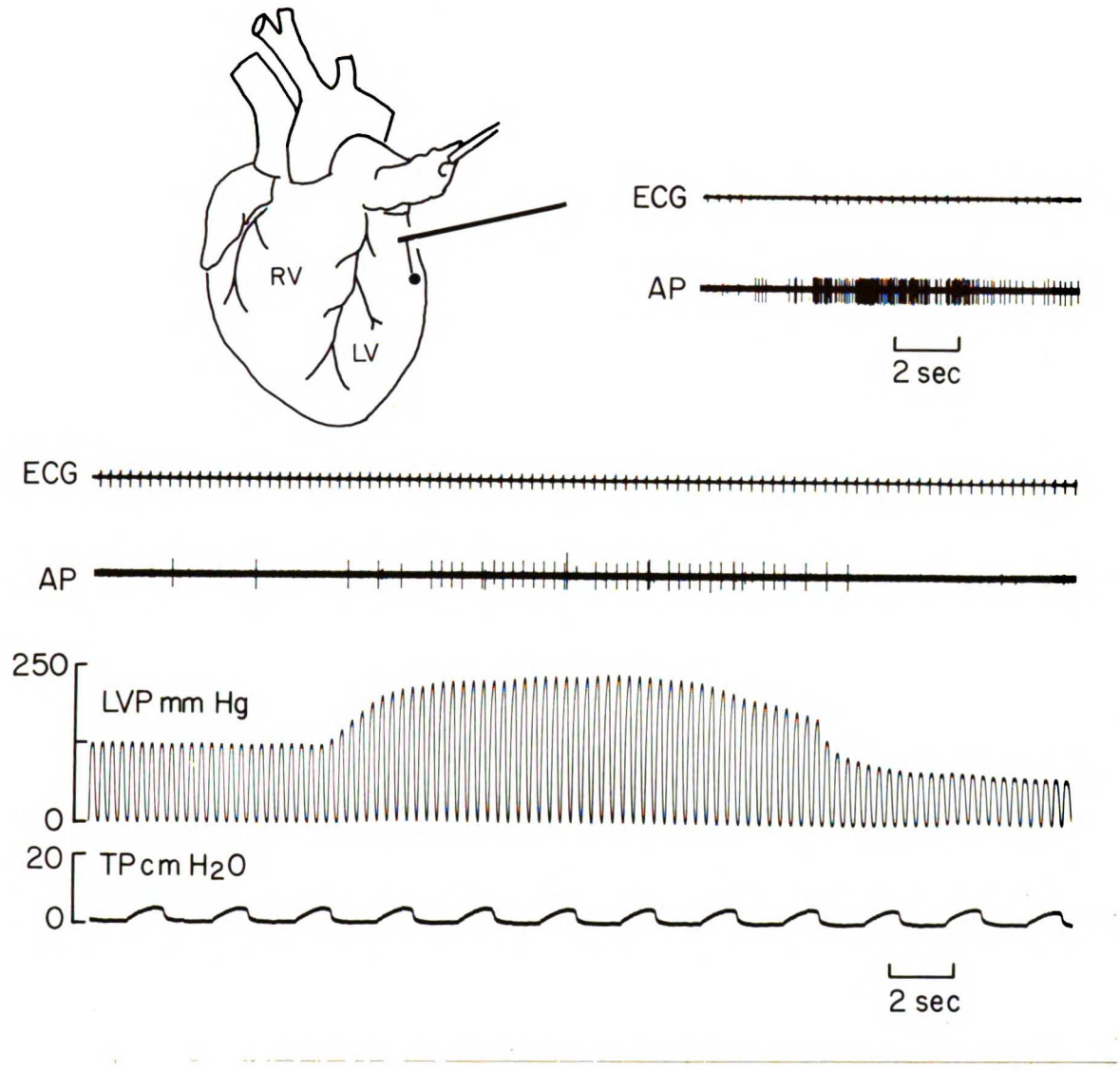

Fig. 15. Stimulation of a sympathetic afferent fiber from the left ventricle by probing, and by increased pressure. Fiber conduction velocity was 8.6 m/sec. AP, action potentials recorded from the upper left thoracic sympathetic chain; LVP, left ventricular pressure; TP, tracheal pressure, positive pressure ventilation. Above, bursts of impulses evoked by stroking the ending with a Frey's hair after the heart stopped. Below, stimulation of the same fiber by an increase in left ventricular pressure, produced by occluding the descending thoracic aorta just above the diaphragm.

fired with a maximal impulse activity of only 2.33 imp/sec (SE±0.33). Some fibers fired with one impulse per heart beat whereas others fired with only a fraction of the heart beats. Because left atrial pressure declined from its peak value during the occlusion, I could not say whether the fibers showed adaptation or not.

RESPONSE TO CHEMICAL AGENTS

Apart from certain differences to be discussed later, the endings I have described appear generally similar to those described by Malliani et al. (Malliani et al., 1973; Lombardi et al., 1976; Malliani and Pagani, 1976). I therefore think that my sample of endings was a reasonably representative one with which to make the next step in my investigation: namely an examination of the response of sympathetic afferent endings in the thorax to bradykinin (BK) and prostaglandin E_1 (PGE_1). Sensitization or stimulation of sympathetic afferent nerve endings by products of tissue damage may be an important component in cardiac pain, and Staszewska-Barczak et al. (1976) reported that topical application of BK (0.02-5 μg in 1 ml saline) to the epicardial surface in lightly anesthetized dogs produced an excitatory nociceptive cardiac reflex with the afferent pathway in the sympathetic nerves. Before I investigated the afferent mechanism involved in the nociceptive reflex described by Staszewska-Barczak et al. (1976)

I first had to determine if epicardial application of BK produced reflex effects in cats similar to those described in dogs.

Reflex Cardiovascular Effects of Bradykinin

My experimental preparation was primarily set up for the recording of sympathetic afferent nerve activity, so that a proportion of the afferent fibers had been sectioned; moreover the level of anesthesia was deep (the corneal reflex was abolished, which prevents manifestation of a painlike pseudoaffective response involving higher centers (Ferreira et al., 1973). Even so, when I applied solutions of BK to the surface of the heart in cats, I observed changes in arterial blood pressure similar to those reported by Staszewska-Barczak et al. (1976). I compared the changes in blood pressure evoked by application of BK to the surface of the heart (45 applications in 27 cats) with changes in blood pressure evoked by application of BK to the pleura some distance away from the heart (45 applications in 28 cats). The results are as follows:

Application of 1 μg BK (in 1 ml saline) to the surface of the heart produced pressor reflexes in 33 of 45 applications, the average increase in mean arterial pressure being 16.6 mmHg (SE±1.29). The response was relatively slow in onset ($\bar{X}$ = 16.6 sec, SE±1.29) and reached a peak

in 35.4 sec (SE±1.8). An example is given in Fig. 16.

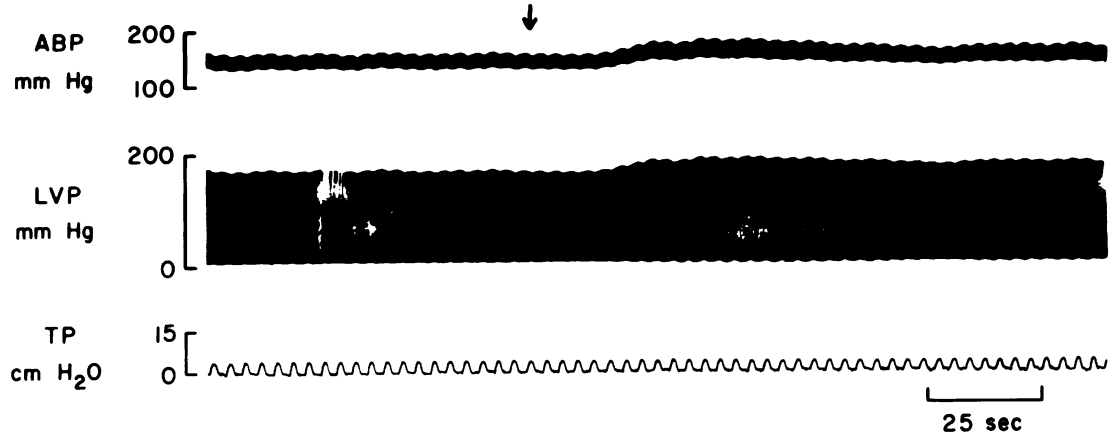

Fig. 16. Increase in arterial blood pressure produced by application of 1 ml BK (1 μg/ml) to the ventricular epicardium. ABP, arterial blood pressure, recorded from the aortic arch; LVP, left ventricular pressure; TP, tracheal pressure. Cat, open chest, positive pressure ventilation. BK was applied at arrow.

On the other hand, application of 1 μg BK to the pleura away from the heart produced pressor reflexes in only 11 of 45 applications. Absence of response did not seem to be related to site of application. The response was significantly much smaller (5.3 mmHg, SE±0.68) and was slow in onset (21.2 sec, SE±4.32).

Thus, by applying BK to the epicardium in cats, I was able to obtain a reflex increase in arterial blood pressure comparable to that observed by Staszewska-Barczak et al. (1976). These authors found that PGE_1 potentiated the

hypertensive effect of BK, but before I proceeded to determine whether PGE_1 potentiated the effect of BK on sympathetic afferent endings in the heart, I first had to examine the effect of these agents alone.

Response to Bradykinin

I applied solutions of BK (1 µg/ml) to 129 sympathetic afferent endings. This concentration was used by Staszewska-Barczak et al. (1976), and is within the range of BK concentrations which occurs naturally in the ischemic myocardium (Furukawa et al., 1969; Kimura et al., 1973). About half (51.2%) of the endings were located inside the pericardial sac, the remainder (48.8%) were located in the viscera or pleura outside the heart. In experiments on all but two of the endings located inside the pericardial sac BK was applied as a drip. In the case of extracardiac endings I applied BK by filter paper or drip in an approximately equal number of experiments.

Since BK was not injected into the blood stream but was applied directly to the receptor site, it was possible that the endings were stimulated either chemically by the solvent (0.9% NaCl-solution) or mechanically by the application itself rather than by the chemical action of BK. To investigate this possiblity, I applied 0.9% NaCl-solution by topical drip or by filter paper to a number of

sympathetic afferent nerve endings. Most endings (26/39) were not stimulated at all by topical application of saline. In 10/39 experiments, however, the results suggest that the endings were stimulated mechanically. There was a brief increase in activity (2-10 imp/sec) lasting 3-6 seconds. This is well demonstrated by a record shown in Fig. 19, in which the brief, initial excitation of the ending was due to mechanical effects. I subtracted the initial increase in activity when I evaluated their response to BK. Finally, in experiments on three endings, application of filter paper soaked in saline caused a prolonged low-frequency discharge; results of these experiments were discarded.

Topical application of 1 μg/ml BK stimulated 115 of the 129 endings (89.1%). After a brief latency, impulse activity increased to a peak value or a plateau before slowly returning to the resting level. The location of the afferent endings and the characteristics of the response are shown in Table 3. Ventricular endings were most sensitive to BK and, compared with endings in the pulmonary veins, aorta and branches or overlying pleura, fired with a significantly shorter latency, had a shorter time to peak response and greater maximal frequency ($P < 0.05$). There were no observable differences between the responses of endings in other locations.

Table 3. Location of sympathetic afferent nerve endings and characteristics of the afferent response evoked by topical application of BK (1 μg/ml). Numbers in parentheses are standard errors.

Location of Endings	# of Ends	Ends Stimulated	Latency (sec)	Maximal Response		Maximal Change in Freq (imp/sec)	Duration (sec)
				Time (sec)	Freq (imp/sec)		
Cardiovascular							
Cardiac							
Ventricles	26	26	12.2 (1.98)	33.3 (3.6)	9.36 (1.48)	8.64 (1.49)	102.9 (11.3)
Left atrium	6	6	9.2 (2.19)	38.0 (5.51)	7.67 (3.33)	6.58 (2.98)	102.8 (13.3)
Great Vessels							
Pulmonary veins	25	21	22.4 (2.53)	47.9 (4.32)	5.55 (0.54)	4.60 (0.54)	95.0 (9.87)
Pulmonary artery	9	7	10.9 (2.43)	37.0 (3.38)	5.96 (1.58)	4.80 (1.49)	103.9 (19.6)
Aorta and branches	9	7	26.1 (2.34)	52.2 (7.25)	5.4 (1.06)	4.71 (1.11)	104.7 (18.4)
Investing Tissue							
Pericardium	8	8	11.5 (4.83)	36.0 (4.77)	3.94 (0.53)	3.10 (0.41)	63.9 (11.5)
Pleura	26	23	15.1 (3.26)	33.4 (3.74)	6.86 (0.91)	6.34 (0.87)	103.9 (12.8)
*Aorta and Branches or Overlying Pleura	20	17	25.9 (7.35)	55.4 (8.53)	4.77 (0.91)	4.38 (0.91)	88.0 (10.7)

*In the case of aortic endings, the pleura was removed except when marked with an asterisk.

There were three major patterns of response to BK: a discharge with a cardiac rhythm, a continuous low frequency discharge and a high frequency discharge, either irregular or continuous. The incidence of the different firing patterns of endings in different locations is shown in Table 4.

Thirty-two of the 129 endings fired 1-2 (occasionally 3) impulses per heart beat with the impulses in any one ending always occurring at the same phase of the cardiac cycle (Fig. 17). Average increase in frequency of these endings was 4.55 imp/sec (SE±0.34).

Sixty-two of 129 endings responded to BK with a low frequency discharge that had no consistent relation to the cardiac cycle (Fig. 18). Maximal increase in frequency of these endings was 4.89 imp.sec (SE±0.29). Many of these endings fired initially with a cardiac rhythm, but as firing frequency increased to more than one imp/cardiac cycle, the discharge became continuous and lost its phasic relation to the cardiac cycle. The continuous nature of the discharge suggests that the endings were stimulated chemically rather than mechanically.

However, in the case of 21 endings there seemed to be no doubt at all that the endings were stimulated directly by BK. The response was often divided into two phases (Fig. 19). Early in the response average impulse activity

Table 4. Location of sympathetic afferent nerve endings and pattern of discharge evoked by topical application of BK (1 μg/ml).

Location of Endings	Number of Endings	Endings Stimulated	Pattern of Discharge		
			Cardiac Rhythm	Low Freq Continuous Discharge	High Freq Discharge
Cardiovascular Endings					
Cardiac endings					
Ventricles	26	26	9	9	8
Left atrium	6	6	3	1	2
Endings in great vessels					
Pulmonary veins	25	21	9	11	1
Pulmonary artery	9	7	4	2	1
Aorta and branches	9	7	0	5	2
Endings in Investing Tissue					
Pericardium	8	8	1	7	0
Pleura	26	23	4	15	4
*Endings in Aorta and Branches or Overlying Pleura	20	17	2	12	3

*In the case of aortic endings, the pleura was removed except when marked with an asterisk.

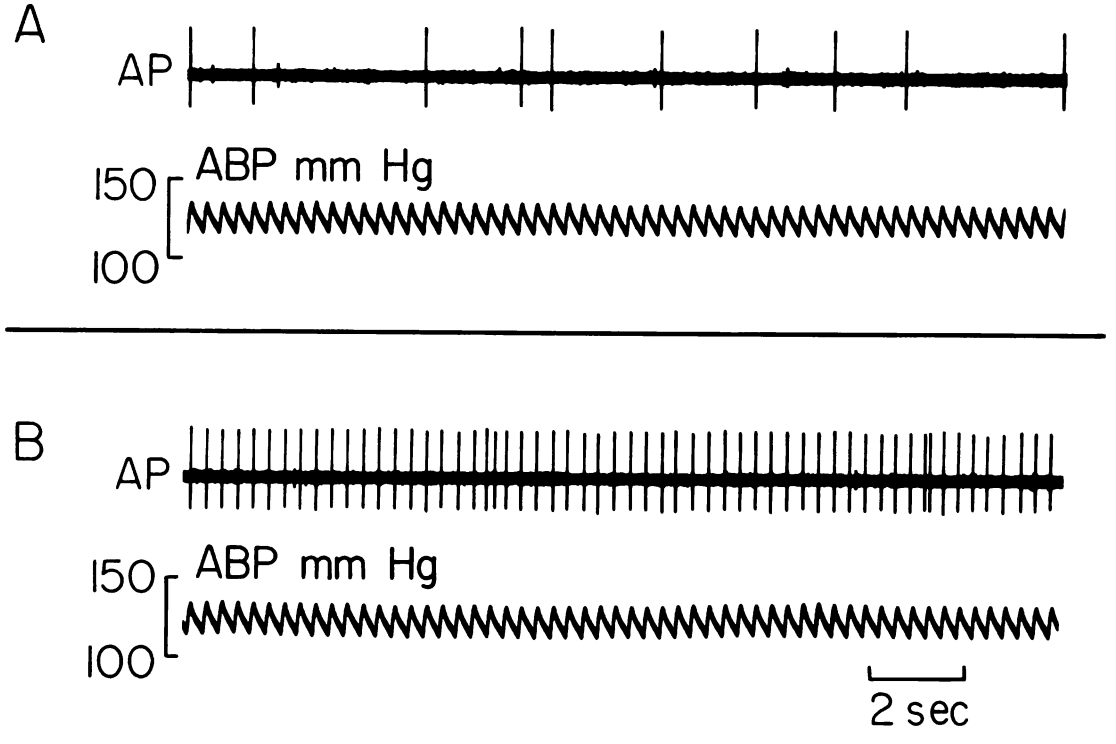

Fig. 17. Excitation of a sympathetic afferent ending from the left ventricle by topical application of BK. Impulses were recorded from a filament of the left sympathetic chain below the third white ramus; fiber conduction velocity was 6.2 m/sec. AP, action potentials; ABP, arterial blood pressure recorded from the aortic arch. The blood pressure was 135/115 mmHg. A, control activity. B, impulse activity after 1 ml BK solution (1 µg/ml) had been dripped onto the ending. The latency of the response was 14 sec; firing at the peak of the response averaged 4.3 imp/sec. Stimulation lasted ca. 4 min. This fiber fired with impulses in systole.

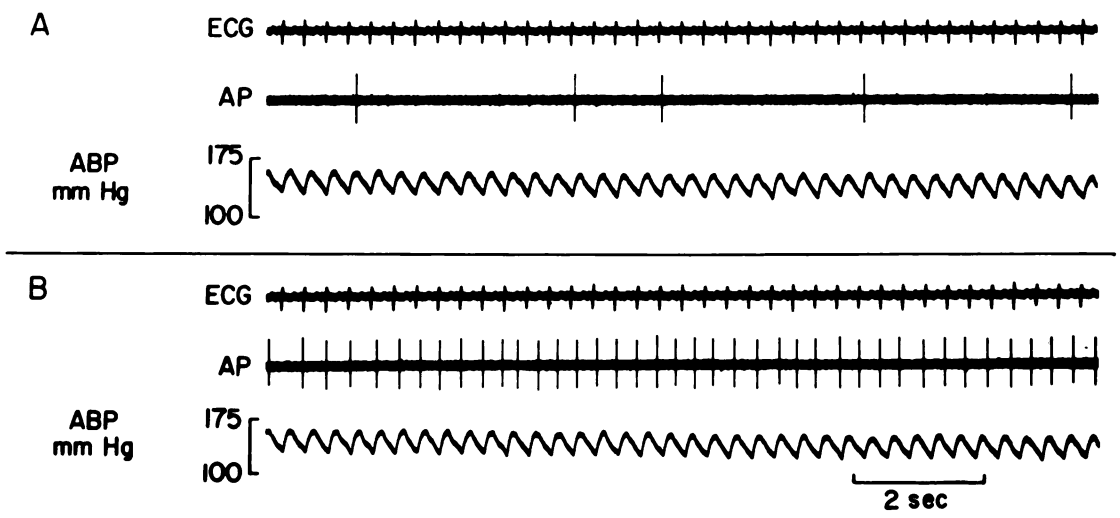

Fig. 18. Excitation of a sympathetic afferent ending in the pericardium by topical application of BK. Impulses were recorded from a filament of the left sympathetic chain below the fourth white ramus; fiber conduction velocity was 6.6 m/sec. AP, action potentials; ABP, arterial blood pressure recorded from the aortic arch. A, control activity. B, 20 sec after a small square of filter paper soaked in BK solution (1 μg/ml) was applied to the receptive site. Latency of the response was 15 sec; firing at the peak of the response was 3.7 imp/sec; stimulation lasted 45 sec. Note that impulses had an irregular relation to the cardiac cycle.

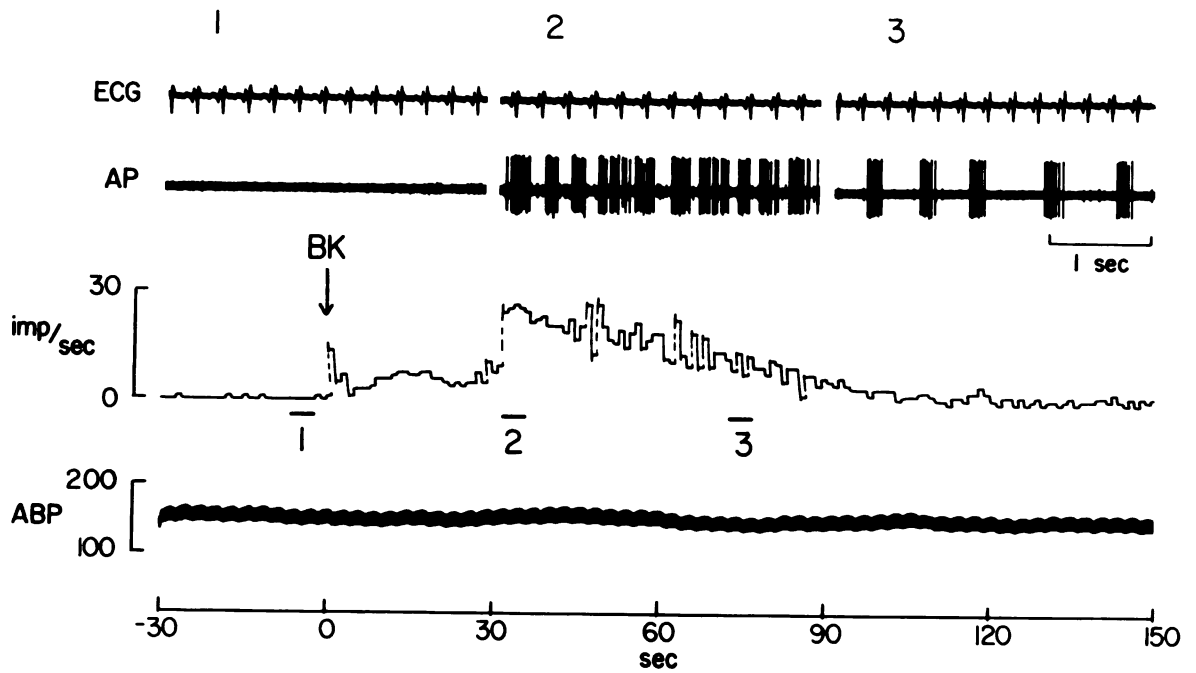

Fig. 19. Excitation of a sympathetic afferent ending in the left ventricle by epicardial application of 1 ml BK (1 μg/ml). Impulses were recorded from a filament of the left sympathetic chain below the third white ramus. Imp/sec, ratemeter recording of impulse activity. Panels 1, 2 and 3 above ratemeter trace show activity recorded at times indicated by bars. AP, action potentials; ECG, electrocardiogram; ABP, arterial blood pressure.

was 3-8 imp/sec, and the discharge either had a cardiac rhythm, or had no consistent relation to the cardiac cycle. After a variable interval (10-30 sec), however, impulse activity suddenly increased to 9-25 imp/sec ($\bar{X}$ = 16.7 imp/sec, SE±1.33), the endings either discharging continuously at high frequency or with high frequency bursts unrelated to cardiac or respiratory events (Fig. 19). The bursting pattern of discharge (Fig. 19, 2) sometimes continued for as long as 2-3 min; the number of spikes per bursts becoming less, and the interval between bursts becoming longer as impulse activity returned to control levels. Endings responding in this way were by far the most chemically sensitive endings, and many of them (10/21) were on the heart (see Table 4). It seems likely that endings of this type provide the afferent input of the nociceptive pressor reflex evoked by epicardial application of BK.

Maximal response to BK and size of the afferent fiber. To determine whether the response to BK was related to the diameter of the afferent fiber I plotted the maximal frequency of discharge against the conduction velocity of the fiber (Fig. 20). Although there was no statistical evidence of a correlation of conduction velocity and intensity of response to BK, nonetheless, the 14 fibers with conduction velocities 10 m/sec or more had maximal responses to BK of less than 6 im/sec, whereas of the eight fibers with the

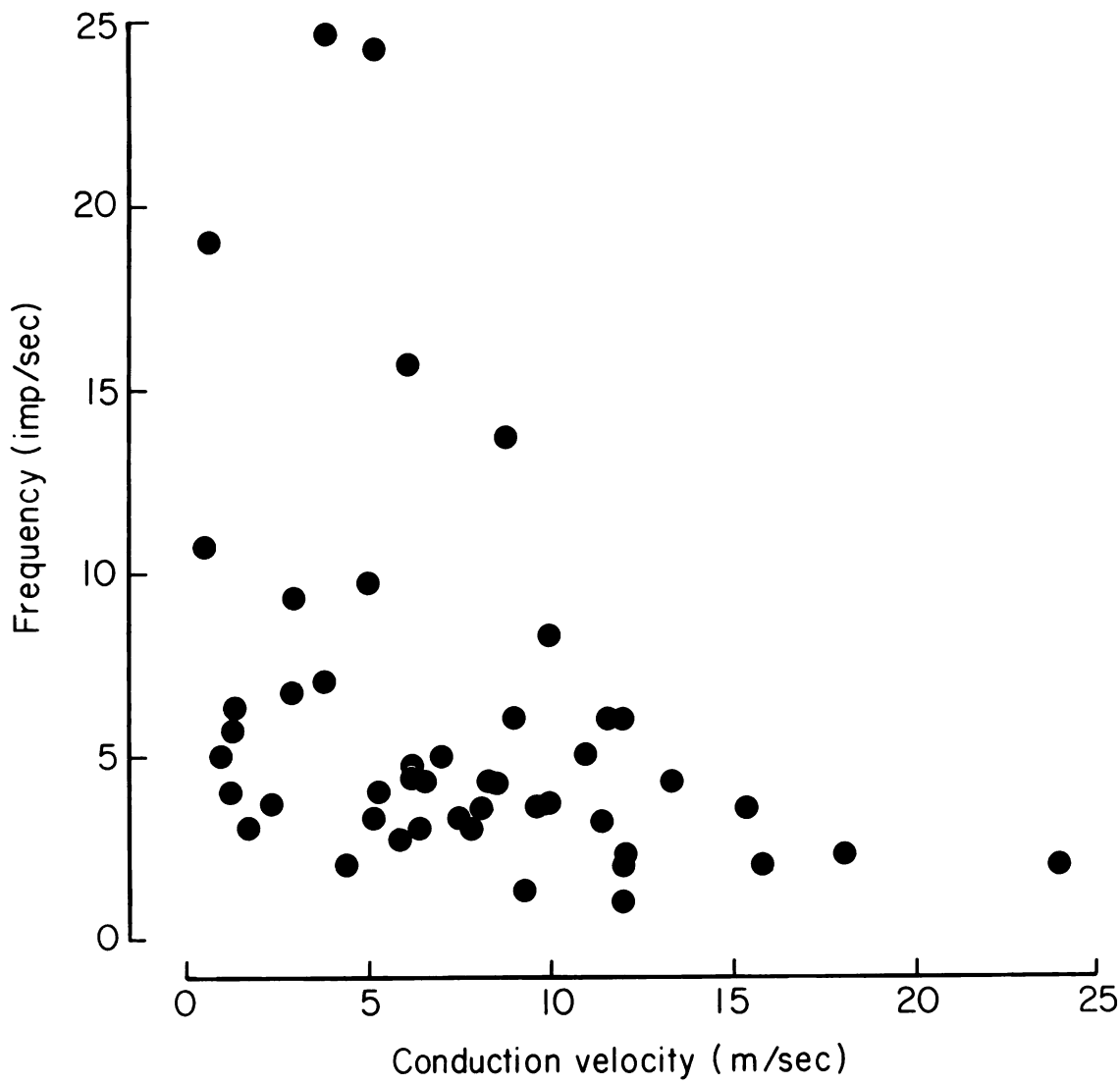

Fig. 20. Maximal increase in impulse activity and the conduction velocity of the afferent fiber. Frequency, maximal increase in impulse activity (counted over a 3-second interval) produced by topical application of 1 µg/ml BK.

greatest response to BK (increases of 9.2-24.7 imp/sec), all but one had a conduction velocity of less than 6 m/sec.

Tachyphylaxis to repeated application of BK. Although Uchida and Murao (1974a) and Nishi et al. (1977) did not describe tachyphylaxis in their studies of the effect of BK on sympathetic afferent nerve endings, I found quite early in my experiments that when I applied BK repeatedly to the same ending, the response became progressively smaller with each application. I therefore examined the phenomenon of tachyphylaxis systematically in observations on 20 endings in various locations, and I applied BK (1 μg/ml) three times to the same ending at 5- to 20-minute intervals.

All endings, no matter where located, showed tachyphylaxis. An example is given in Fig. 21, which shows the responses of a left ventricular ending to three consecutive applications of BK at 15-minute intervals; the responses had progressively longer latencies and lower maximal frequencies (Fig. 22).

BK-application to multiple endings. I selected 10 afferent fibers with more than one ending apiece (2-3 endings each) and I carefully determined the location of the endings by probing with a bristle. I then applied BK (1 μg/ml) to each of the endings in turn. On most occasions I applied a small square of filter paper soaked in the BK-

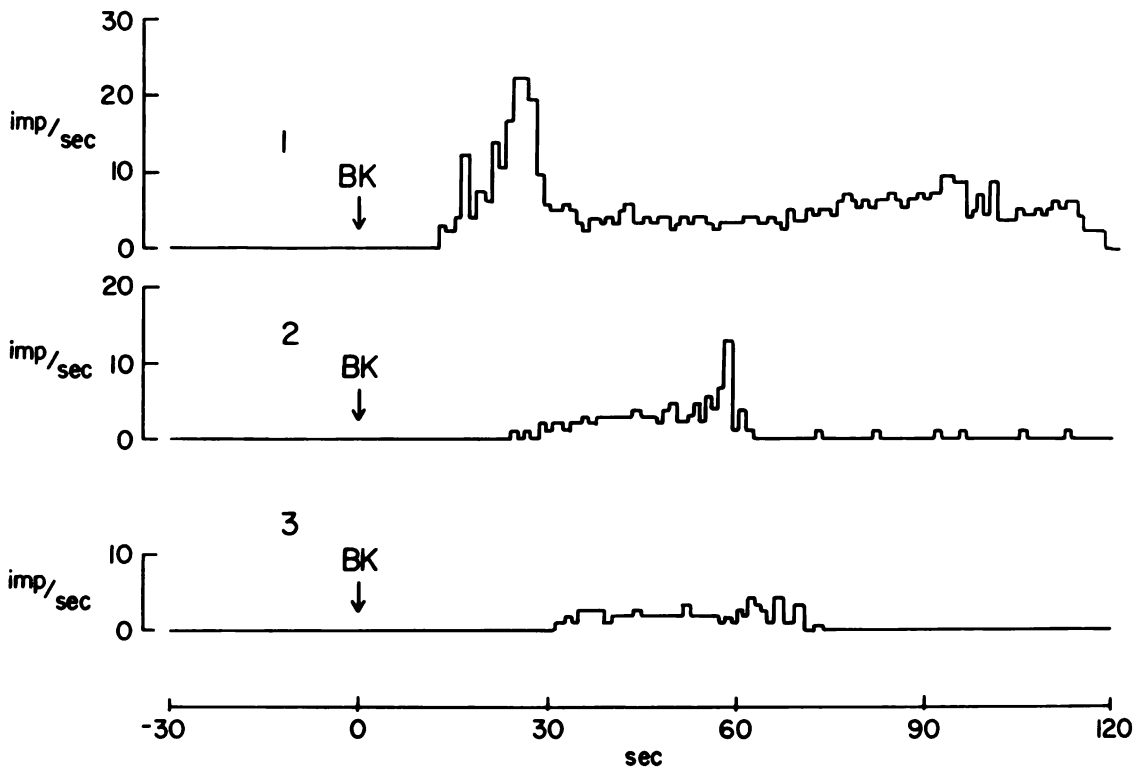

Fig. 21. Tachyphylaxis to repeated application of BK. Imp/sec, activity of a sympathetic afferent fiber with ending on the left ventricle. Impulse activity was recorded from a filament of the left sympathetic chain below the 3rd white ramus; fiber conduction velocity was 0.64 m/sec. In 1, 2 and 3 one ml BK (1 μg/ml) was applied to the epicardium at arrows; 15 minutes between each application.

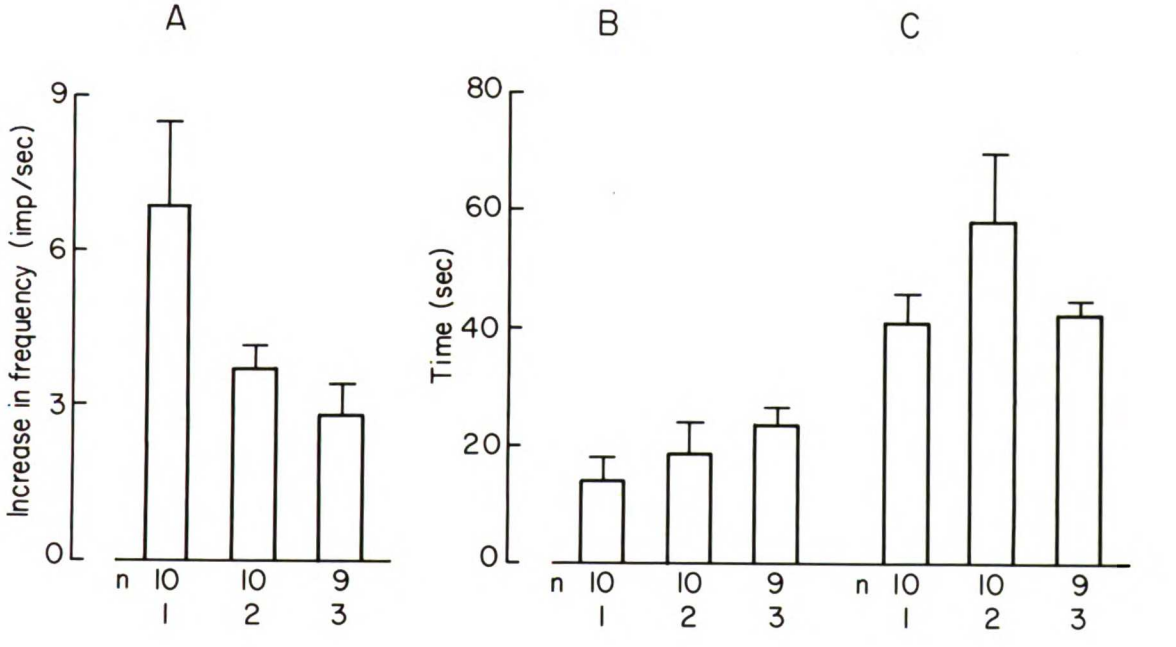

Fig. 22. Tachyphylaxis to repeated application of BK. Average response of 10 sympathetic afferent fibers to repeated application of BK (1 μg/ml) at 15-minute intervals. A, Frequency, maximal increase in impulse activity counted over 3 seconds at peak response; significantly smaller for second application. B, latency of the response; significantly longer for second application. C, time interval from beginning of application to maximal response. 1, 2 and 3 are first, second and third applications of BK. Statistical variation is indicated by standard error. Student paired T-test, $P < 0.05$.

solution to the region of the ending and a similar square of filter paper soaked in BK to a "neutral" spot between two endings of the same afferent fiber. Application of BK to endings of the same afferent fiber some distance apart evoked a response in the fiber, whereas application of BK to a neutral spot (which did not respond to probing), had no effect. Results obtained from an experiment of this type is shown in Fig. 23.

Application of BK to the two endings of a common parent fiber often evoked responses whose latencies of onset, peak frequencies and duration of response varied considerably. For example, application of BK to one ending evoked a peak frequency of discharge of 6.3 imp/sec, whereas subsequent application of BK to another ending of the same afferent fiber evoked a vigorous response of 13.7 imp/sec. Similarly, another multiterminal fiber responded with peak frequencies of 5.3 and 16 imp/sec, respectively when BK was applied to its two endings in turn.

Another striking feature of the response of multiple endings to BK was that after the response of one ending successively diminished with repeated application as tachyphylaxis developed, a high frequency discharge could then be evoked by applying BK to the second ending. For example, BK was applied at 5-minute intervals to one ending, evoking maximum responses of 2.57, 1.37 and 0.4 imp/sec, respectively.

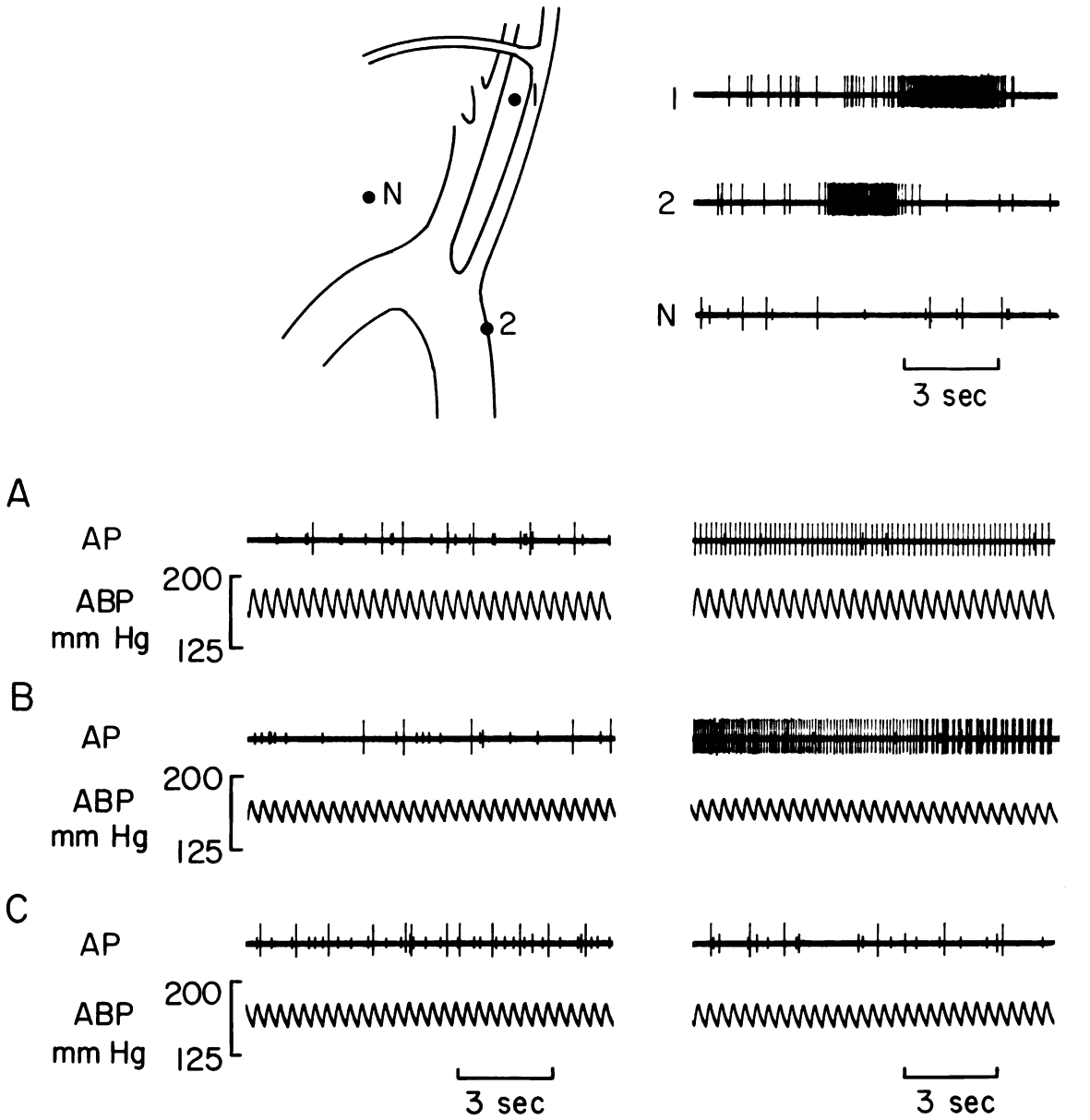

Fig. 23. Mechanical and chemical excitation of a sympathetic afferent fiber with two endings. Impulse activity was recorded from a filament of the left sympathetic chain below the 4th white ramus. Fiber conduction velocity was 8.8 m/sec. The fiber had two endings (1 and 2). Stroking the endings with a bristle evoked bursts of impulses (large spikes) shown in panels 1 and 2 on the right above; stroking a neutral spot (N) on the pleura had no effect. Lower half of the figure shows the response to separate application of BK to each ending and to the neutral spot. Left panels, control activity; right panels, impulse activity after application of BK (1 μg/ml). A, application to spot 1. B, application to spot 2. C, application to the neutral spot. AP, action potentials; ABP, arterial blood pressure, recorded from the aortic arch. The small spikes are action potentials recorded from a different fiber in the same filament. The location of its terminals could not be determined.

BK was then applied to the second ending, from which it evoked a vigorous response of 5.67 imp/sec. In other words, tachyphylaxis seems to be a property of the ending and not of the fiber.

Response to Prostaglandin E_1

I applied solutions of PGE_1 to a total of 71 sympathetic afferent nerve endings. Following the protocol of Staszewska-Barczak et al. (1976), I applied 0.1 µg/ml PGE_1 to 28 sympathetic afferent nerve endings located in the heart or great vessels (ventricles 13, pulmonary veins 2, aorta 6), pericardium (2) and pleura (5). I also applied PGE_1 in 100 times this concentration (10 µg/ml) to 43 afferent endings located as follows: ventricles 13, left atrium/pulmonary veins 16, pulmonary artery 2, aorta 9, pericardium 1 and pleura 2. The protaglandin solution was applied by topical drip to 63/71 of the nerve endings, and by filter paper to the remaining eight endings.

To determine if the trace amount of ethanol present in the solutions of PGE_1 had an effect by itself, I applied an appropriate solution of ethanol in saline (10 ppm) to eight sympathetic afferent nerve endings. This had no effect on impulse activity, nor when applied for 20 minutes did it potentiate the response to BK (see below).

Only nine of 71 endings (12.7%) were stimulated by

PGE_1; three of 28 endings were stimulated by 0.1 μg/ml and six of 43 endings by 10 μg/ml. However, the majority of endings that were stimulated by PGE_1 (seven of nine endings) were located in the ventricle. Thus, 7/26 ventricular endings (27%) were stimulated, whereas only 2/45 endings (4.4%) located elsewhere, which suggests that cardiac fibers are particularly sensitive to PGE_1.

PGE_1 evoked each of the three major response patterns described on page 75. Three endings fired with a cardiac rhythm at 3.3 imp/sec, three endings fired with slightly higher impulse activity that had no consistent relation to the cardiac cycle, and three endings fired with high frequency bursts or with high frequency continuous discharge (Fig. 24). Of the nine endings stimulated by PGE_1, the latency of the response ranged from 9-100 sec ($\bar{X}$ = 21.4, SE±10.1), the peak frequency of the response ranged from 2.6-17.5 imp/sec ($\bar{X}$ = 6.47, SE±1.94) and the duration ranged from 12-87 sec ($\bar{X}$ = 55.7 sec, SE±11.3). Application of PGE_1 appeared to have no effect on blood pressure, so the change in impulse activity produced by PGE_1 was not secondary to mechanical changes in the heart and great vessels.

Interaction of Bradykinin and Prostaglandin E_1

Having examined the independent effect of BK and PGE_1 on sympathetic afferent nerve endings, I next examined the

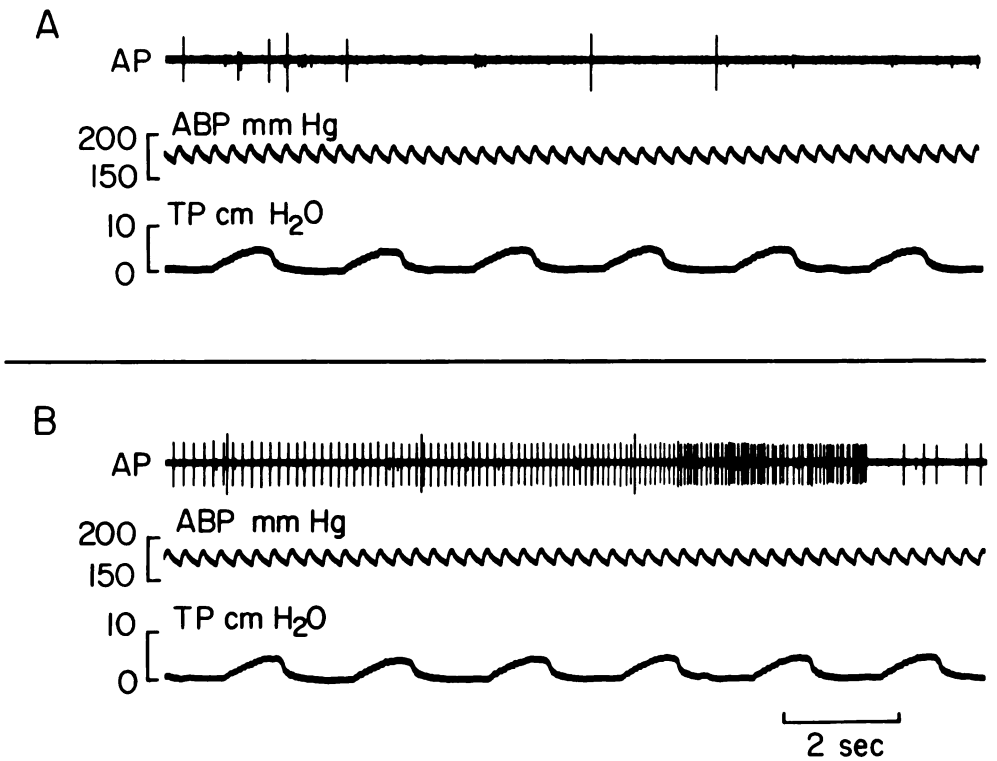

Fig. 24. Excitation of a sympathetic afferent ending from the pleura (small spikes) by topical application of PGE_1. Impulses were recorded from a filament of the 3rd white ramus. AP, action potentials; ABP, arterial blood pressure, recorded from the aortic arch. TP, tracheal pressure; open chest, positive pressure ventilation. A, control activity. B, impulse activity after 1 ml PGE_1 (10 µg/ml) was dripped onto the pleura. Latency of the response was 21 sec; firing at the peak of the response averaged 8.6 imp/sec; stimulation lasted 48 sec. The large spikes are action potentials recorded from a different fiber in the same filament. The location of its terminals could not be determined.

combined effects of these two substances. Specifically, I wished to determine whether the effect of BK was potentiated by PGE_1, as Staszewska-Barczak et al. (1976) suggested in their reflex study. This proved to be more difficult than I had anticipated, and I changed the experimental protocol several times before I found a satisfactory one.

First I adopted a simple protocol used in the reflex study of Staszewska-Barczak et al. (1976). In experiments on nine sympathetic afferent endings I compared the effect of applying BK alone (one ml saline containing 1 µg BK) with the effect of applying BK and PGE_1 together (1 µg BK and 10 µg PGE_1 in one ml saline) 15-46 minutes later. Eight of the endings were located inside the pericardial sac (left ventricle 4, right ventricle 2, pulmonary vein/atrial junction 2) and one was in the pleura. One had a C-fiber with a conduction velocity of 1 m/sec, while the others had small myelinated fibers (3.7-9.6 m/sec). In the event, although Staszewska-Barczak et al. (1976) had successfully used this protocol to demonstrate that the hypertensive effect of BK was potentiated by PGE_1, in my experiments I found that the intensity of stimulation produced by BK and PGE_1 together was no greater than that produced by BK alone (see Table 5).

A possible explanation for my failure to demonstrate a potentiating effect of PGE_1 may have been that the tissue

Table 5. Impulse activity evoked by application of BK (1 µg/ml in 1 ml saline) or by simultaneous application of BK and PGE_1 (1 µg BK and 10 µg PGE_1 in 1 ml saline) to nine sympathetic afferent nerve endings. Application of PGE_1 (10 µg/ml) alone had no effect. Standard error in parenthesis.

Drug	Control Frequency (imp/sec)	Maximal Increase in Frequency (imp/sec)	Average Increase in Frequency (imp/sec)
BK	0.61 (0.18)	6.18 (2.18)	2.95 (0.69)
BK + PGE_1	0.89 (0.26)	5.96 (2.59)	2.45 (0.92)

(The results are not significantly different. Student T-test for paired data.)

levels of prostaglandins were already quite high. Prostaglandins are readily released by the tissues in response to a number of stimuli, particularly trauma and mechanical stimulation (Piper and Vane, 1971), and my cats had been subject to considerable surgical interference. Hence, the first application of BK may already have produced a maximal response, so that no further increase could be expected from addition of exogenous PGE_1. I therefore needed a preparation in which endogenous prostaglandins would not be formed and released during the course of the experiment.

In the next series of experiments I therefore followed

another of the protocols of Staszewska-Barczak et al. (1976) and examined the response to BK after the formation and release of endogenous prostaglandins had been inhibited by administration of indomethacin and compared this response with that evoked by BK after the receptive site had been primed with PGE_1. Following the advice of Dr. John McGiff (University of Tennessee), I infused 2 mg indomethacin/kg body weight and assumed that its inhibiting effect lasted two hours. In these experiments, instead of giving the prostaglandin as a single application (see above), I followed the technique of Juan and Lembeck (1974) and primed the receptor site with 0.1 µg/ml PGE_1 (0.2-0.5 ml/min) for 20 minutes. These authors had found that the potentiating effect of PGE_1 was cumulative, and that maximal potentiation was achieved only after PGE_1 was superfused or perfused for at least 20 minutes.

I used this protocol to examine 10 cardiovascular endings (left ventricle 6, pulmonary vein 2, aorta 2). In the majority of experiments (8/10) the changes in impulse activity were in the same direction as the reflex changes in blood pressure reported by Staszewska-Barczak et al. (1976). Thus, PGE_1 (0.1 µg/ml) alone had no effect on impulse activity but it did increase the response to BK. However, for the group of endings as a whole, the effect of PGE_1 on maximal increase in frequency and average increase in frequency were not statistically significant at a 5%

level (Student T-test for paired data) (see Table 6, II).

Table 6. Impulse activity evoked by application of BK (1 μg/ml) I, before and after infusion of indomethacin (2 mg/kg), and II, after infusion of indomethacin, and after priming the receptive area with PGE_1 (0.1 μg/ml, 0.2-0.5 ml/min) for 20 minutes. Application of PGE_1 (0.1 μg/ml) alone had no effect. Standard error in parenthesis.

Group	Treatment	Control Frequency (imp/sec)	Maximal Increase in Frequency (imp/sec)	Average Increase in Frequency (imp/sec)
I N=4	BK	0.81 (0.31)	2.57 (0.20)	1.32 (0.19)
	BK after indo	0.47 (0.17)	0.79 (0.28)	0.29 (0.08)
II N=10	BK after indo	0.51 (0.20)	1.36 (0.47)	0.75 (0.25)
	BK after PGE_1	0.80 (0.28)	2.23 (0.24)	1.10 (0.26)
			(P > 15%)	(P > 50%)

In experiments on four of these 10 endings I was able to examine the effect of indomethacin on the response to BK. As can be seen in Table 6, I, the response to BK, represented as maximal increase in frequency and average increase in frequency, was diminished after indomethacin. However, I was unable to determine if the decrease in the response to BK was due to the indomethacin itself or was a result of the tachyphylaxis associated with repeated application of

BK (see below).

Tachyphylaxis is a prominent feature of the response of cutaneous afferent fibers to BK (Handwerker, 1976). In the course of my experiments on sympathetic afferent fibers I always observed marked tachyphylaxis in the response to BK (pp. 83-84, Figs. 21, 22). I therefore devised a protocol which would enable me to examine the potentiating effect of PGE_1 and which would also take into account the occurrence of tachyphylaxis with repeated applications of BK. The procedure was as follows. I first infused indomethacin i.v. On advice from Dr. Gabor Kaley (New York Medical College), I then increased the dose of indomethacin to 5 mg/kg and assumed that the inhibitory effect lasted throughout the experiment. I then applied BK (1 µg/ml, 0.5-1.0 ml) three times at 15-minute intervals. I next dripped a solution of PGE_1 (0.1 µg/ml, 0.2-0.5 ml/min) on to the receptor site for 20 minutes. Finally I reapplied BK 2-3 times at 15-minute intervals, with continued application of PGE_1 between each application of BK.

This protocol was followed in experiments on 13 sympathetic afferent endings, ten of which were located inside the pericardial sac (left ventricle 4, left atrium/pulmonary veins 6) and three were in the pleura. Individual fibers differed in the pattern of their response to BK; some fired with a cardiac rhythm, others fired with a continuous

discharge and still others fired with high frequency bursts. Regardless of the pattern of the response, however, the intensity of the response became progressively smaller with each successive application of BK after administration of indomethacin. Thus, whereas the first application of BK after indomethacin caused an average increase in maximal frequency of 7.28 imp/sec (SE±2.04), the third application caused an increase of only 3.64 imp/sec (SE±0.96) (see Table 7). I then applied PGE_1 (0.1 µg/ml, 0.2-0.5 ml/min) to the endings for 20 minutes. PGE_1 by itself had no observable excitatory effect at all on these endings, but it did restore their responsiveness to BK (see Figs. 25, 26 and 27). When I reapplied BK after application of PGE_1, maximal increase in impulse activity in these 13 fibers reached 9.93 imp/sec (SE±2.44), which is greater than the response evoked by the first BK-application after indomethacin infusion (7.28 imp/sec, SE±2.04), and significantly greater than the last response to BK before administration of PGE_1 (3.64 imp/sec, SE±0.96).

The response of a single sympathetic afferent fiber is illustrated in Fig. 25. Before administration of PGE_1, topical application of BK (1 µg/ml) produced a continuous discharge with a maximal frequency of 5.0 imp/sec, but when I reapplied BK after treatment with PGE_1, the fiber fired with high frequency bursts of 15 imp/sec.

Table 7. Average response evoked in a group of sympathetic afferent fibers by repeated application of BK after infusion of indomethacin (5 mg/kg). Series A and B, application of BK (1 µg/ml) at 15-min intervals. Between A_3 and B_1, receptive area primed with PGE_1 (0.2-0.5 ml/min, 0.1 µg/ml) for 20 min. PGE_1-priming also continued between B_1 and B_2, and B_2 and B_3. Application of PGE_1 (0.1 µg/ml) alone had no effect. Standard error in parentheses.

Fibers	Control Activity (imp/sec)	Onset (sec)	Time to Peak Response (sec)	Maximal Change in Frequency (imp/sec)	Average Change in Frequency (imp/sec)	Duration (sec)
A. After Indomethacin						
1. 11	0.13 (0.03)	13.1 (2.8)	36.3 (4.2)	7.28 (2.04)	2.78 (0.54)	139.1 (29.4)
2. 13	0.09 (0.02)	12.8 (2.6)	31.3 (6.2)	4.47 (1.03)	1.21 (0.26)	158.1 (51.6)
3. 13	0.08 (0.03)	12.0 (3.64)	36.2 (4.5)	3.64 (0.96)	1.24 (0.33)	137.7 (56.1)
B. After PGE_1-priming						
1. 13	0.38 (0.10)	12.0 (2.65)	33.9 (6.0)	9.93 (2.44)	3.89 (0.73)	161.4 (34.8)
2. 8	0.65 (0.21)	11.1 (2.5)	37.2 (6.1)	9.52 (3.12)	3.34 (0.93)	124.5 (16.7)
3. 4	0.45 (0.31)	19.3 (9.51)	42.0 (13.1)	10.10 (4.59)	3.33 (1.25)	104.5 (5.72)

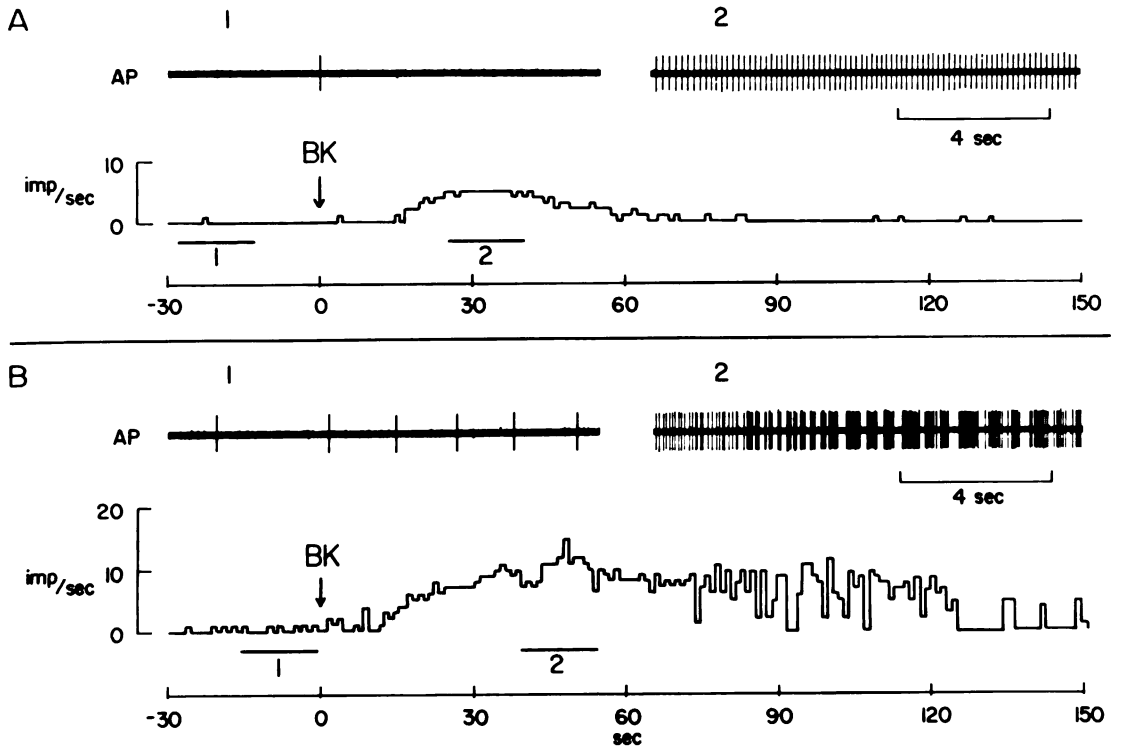

Fig. 25. Impulse activity (imp/sec) of a sympathetic afferent ending from the pleura, recorded from a filament of the left sympathetic chain below the second white ramus; fiber conduction velocity was 0.5 m/sec. A, response of ending to third topical application of BK (1 μg/ml, 1 ml) after infusion of indomethacin (5 mg/kg). Between A and B 0.3 ml PGE_1 (0.1 μg/ml) was applied to the region of the ending each minute for 20 minutes. B, response of the fiber to topical application of BK after PGE_1. Panels 1 and 2 above each ratemeter trace show action potentials (AP) recorded at times indicated by bars.

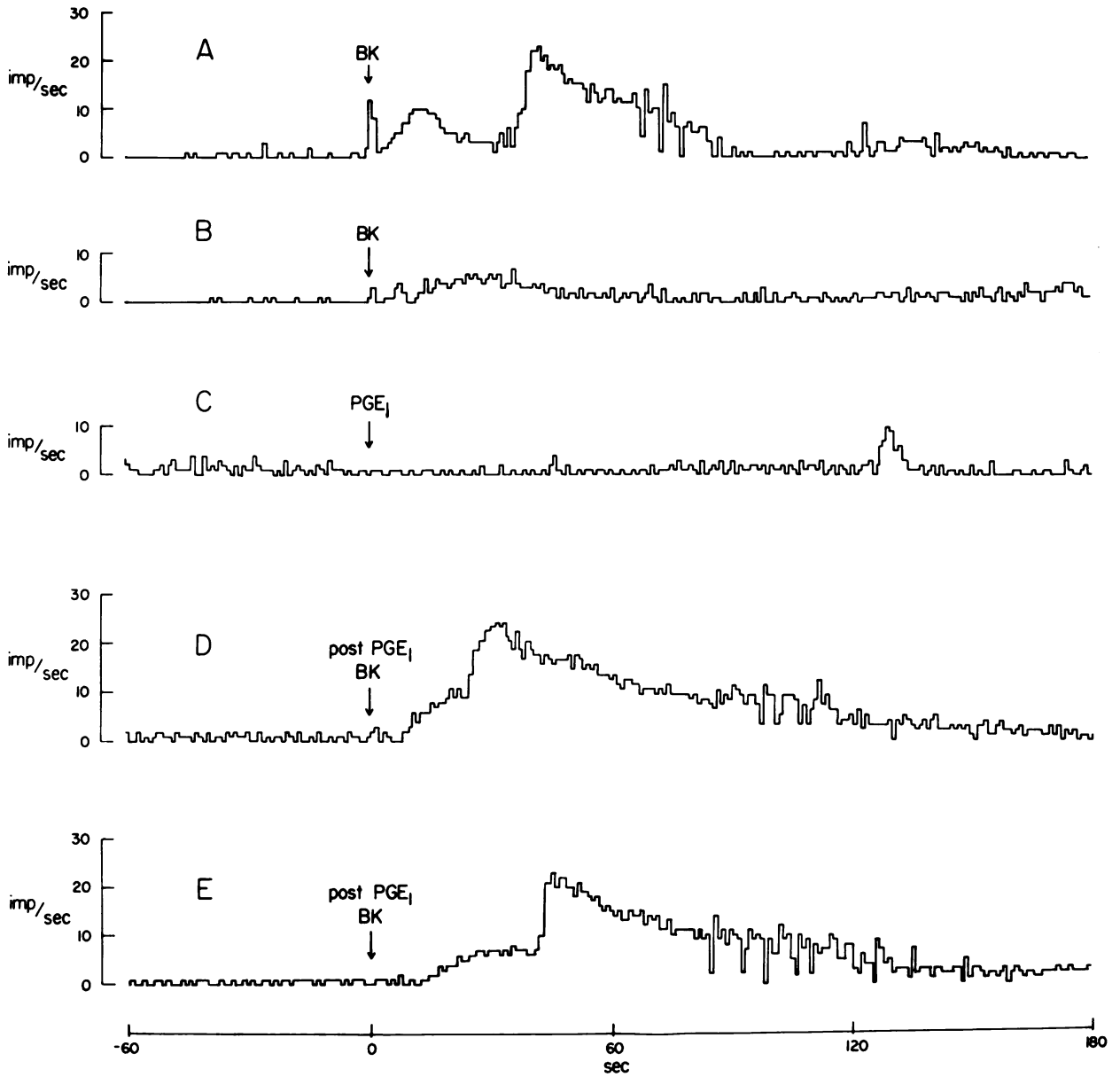

Fig. 26. Impulse activity (imp/sec) of a sympathetic afferent ending (right ventricle), recorded from the left sympathetic chain below the third white ramus; fiber conduction velocity was 5.2 m/sec; cat treated with indomethacin (5 mg/kg i.v.). A and B, response to second and third application of BK (1 µg/ml); interval A-B, 15 minutes. PGE_1 application began at arrow in C: 0.3 ml PGE_1 (0.1 µg/ml), applied to the ending each min for 20 min. D and E, response to subsequent applications of BK (1 µg/ml); interval 15 min. PGE_1 application continued between D and E.

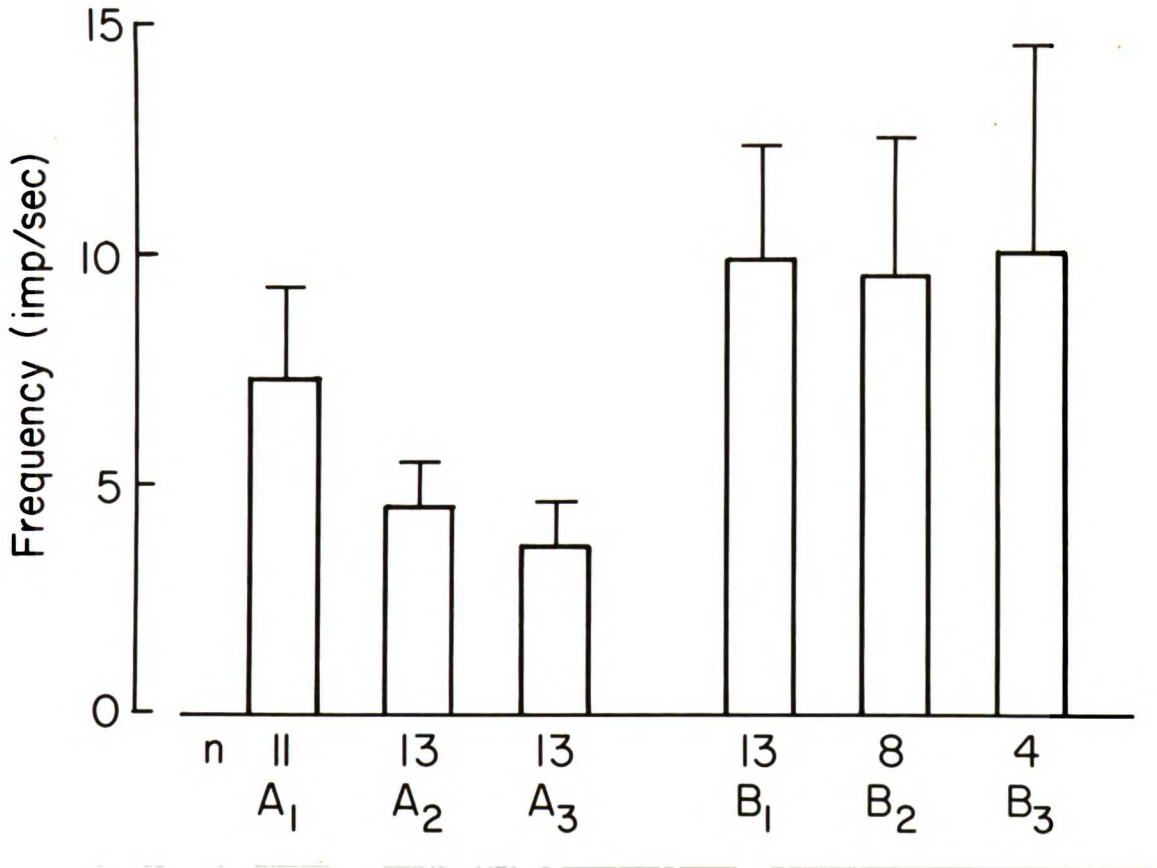

Fig. 27. Average increase in impulse activity (counted over 3 sec at peak response) of a group of sympathetic afferent fibers to repeated application of BK. $\underline{A_1}$, $\underline{A_2}$ and $\underline{A_3}$, response to topical application of $\overline{BK}$ ($\overline{1}$ µg/ml) at 15-min intervals after infusion of indomethacin (5 mg/kg). Between A_3 and B_1 0.2-0.5 ml PGE_1 (0.1 µg/ml) was applied to the ending each minute for 20 minutes. $\underline{B_1}$, $\underline{B_2}$ and $\underline{B_3}$, response to subsequent applications of $\overline{BK}$ (1 µg/ml) at 15-min intervals. PGE_1-treatment is continued between applications.

In experiments on eight of these 13 fibers, the fiber remained alive so that I was able to examine the response to a second application of BK after PGE_1-priming. The results obtained strongly suggest that prostaglandins acted by counteracting the tachyphylaxis to BK. An example is given in Fig. 26. Whereas tachyphylaxis was a prominent feature of the response to BK before PGE_1-application (A and B in Fig. 26), the response to the second application of BK after PGE_1 was only slightly smaller than that evoked by the first application (U and E in Fig. 26), showing very little tachyphylaxis. This is also illustrated in Fig. 27, which shows maximal increase in frequency in all 13 fibers to repeated application of BK after indomethacin and after priming with PGE_1.

In addition, in experiments on four of these 13 afferent fibers, I was also able to give a third application of BK after administration of PGE_1. (The progressive reduction in numbers of fibers from 13 in A_2 to eight in B_2 and four in B_3 (see Table 7) is due to the increasing number of fibers becoming inactive with time (ca. 1.5-2 hours) on the recording electrodes.) Once again, the third application of BK after administration of PGE_1 evoked an afferent response that on average was comparable to that evoked by the two previous applications of BK. Thus, after administration of PGE_1, the response to repeated application of BK showed very little tachyphylaxis.

In these 13 fibers the conduction velocity varied from C-fibers with conduction velocities of 0.5 and 0.64 m/sec to Aδ-fibers with conduction velocities from 5.2 to 11.8 m/sec, and as far as I could determine there was no difference in their qualitative response.

DISCUSSION

GENERAL PROPERTIES

Most investigators agree that sympathetic afferent Aδ fibers from the heart and great vessels have a low tonic discharge (usually less than 1 imp/heart beat) that is regularly related to a given phase of the cardiac cycle: ventricular fibers fire consistently with either ventricular contraction or relaxation (Malliani et al., 1973; Uchida et al., 1974; Uchida, 1975a), fibers from the atria or pulmonary veins fire with either the a, c, or v wave of the atrial pressure pulse (Malliani et al., 1973; Uchida and Murao, 1974c; Lombardi et al., 1976) and fibers from the pulmonary artery or thoracic aorta fire with the upstroke of the pressure pulse (Nishi et al., 1974; Uchida, 1975b; Malliani and Pagani, 1976). This cardiac rhythm was one of the features of the Aδ fibers that persuaded Malliani et al. (1975) that sympathetic afferent fibers are mechanoreceptors which provide the spinal cord with essential information about specific hemodynamic events, and Nishi et al. (1974) even call them "pulmonary arterial baroreceptors".

Although some afferent fibers in my sample were silent

at normal arterial pressure, as described by Ueda et al. (1969) and Uchida (1975b), most were tonically active. However, I found that less than half (45%) had a resting discharge that was regularly related to a given phase of the cardiac cycle, and as many as 36% of the fibers had a resting discharge that had no consistent relation to the cardiac cycle (irregular discharge). Although the high incidence of irregular firing pattern in my sample differs from that in previous studies, several investigators have mentioned in passing that afferent sympathetic fibers can have an irregular discharge. For instance, Malliani et al. (1973) mentioned that atrial and ventricular fibers could spontaneously change their pattern of discharge, Malliani and Pagani (1976) admitted that aortic fibers would occasionally fire with a more irregular discharge, and Uchida (1975a and b) mentioned that 1/4 of Aδ fibers from the right ventricle and 1/6 of aortic fibers fired with an irregular pattern at rest. However, except for Uchida (1975a and b), they gave no systematic description of the incidence of the firing patterns in the sample, and made no reference to the functional implications of the different patterns of control discharge.

I find it particularly interesting that the incidence of the different firing patterns was the same for cardiovascular endings as for endings in investing tissue such as the pericardium and pleura. This suggests that the infor-

mation provided to the spinal cord by an ending in the wall of the left ventricle is similar to that provided by a pleural ending located some distance from a cardiovascular structure. Moreover, since 45% of the sympathetic afferent fibers from the thorax appear to have multiple endings, some distributed to different structures, this is hardly surprising. Thus, under resting conditions, the information conveyed even in fibers with endings in the walls of cardiovascular structures is of a very different order from that supplied by the vagal and carotid sinus nerves, which fire with a pulsatile discharge for each heart beat, providing a frequency analogue of the pressure pulse.

I have examined the control discharge of a large number of afferent fibers from the heart, great vessels and associated connective tissue. The range of frequencies observed in my sample was similar to that observed by Nishi et al. (1974) in fibers from the pulmonary artery, and by Malliani and Pagani (1976) in fibers from the thoracic aorta. Like Malliani and Pagani (1976) I found that Aδ and C-fibers had similar frequencies at rest, and in addition, each type of activity was represented in my small sample of C-fibers. This, combined with the fact that Aδ and C-fibers from the aorta gave a similar response to pressure (Malliani and Pagani, 1976), suggest that the transducer properties of the two groups are generally similar.

The endings of sympathetic afferent fibers were extremely sensitive to touch, which made them easy to locate. Although most investigators have found that a very light stroking of the external surface of the heart or great vessels in the region of the ending evoked high frequency bursts (Ueda et al., 1969; Malliani et al., 1973; Nishi et al., 1974; Uchida et al., 1974; Uchida and Murao, 1974c; Malliani and Pagani, 1976) they did not pursue the functional implications of this finding. I found that even in a thin-walled structure like the left atrium the endings were more sensitive to probing of the outer surface of the atrium than to probing from the inside. It therefore seems reasonable to conclude that afferent nerve endings are located close to the outer surface of the viscera.

Sympathetic afferent fibers are widely distributed throughout the viscera and connective tissue of the thorax, a distribution which was well described in the first general studies in this field (Holmes and Torrance, 1959; Ueda et al., 1969), although subsequent investigators have usually confined their studies to examination of endings in particular structures, such as the aorta, atrium and ventricle. I found that the left sympathetic chain and rami carried fibers whose endings were distributed throughout the left side of the thorax: in the cardiac ventricles, in the left atrium, in the pulmonary artery and veins, in the thoracic aorta and its branches and in connective tissue like the

pericardium and pleura. Certain areas such as the aortic arch, the ventricles and the left atriovenous junctions had a particularly high concentration of endings, but the sympathetic innervation was widespread throughout the various thoracic structures. Thus, the anatomical distribution of sympathetic afferent nerve endings form a striking contrast to that of myelinated vagal afferent endings, which are focal in distribution and have a high concentration in certain key areas such as the atriovenous junctions and the aortic arch while leaving large "silent" areas in between (Coleridge et al., 1973).

Large numbers of nerve endings were in the pericardial sac and pleura, which envelop and bind down the thoracic organs. Although this latter feature was well described in the first electrophysiological studies of sympathetic afferent nerve endings (Holmes and Torrance, 1959; Ueda et al., 1969), it has received little attention in the many studies done within the last decade. When discussing the functional role of sympathetic afferent fibers from the thorax, it should be kept in mind that many fibers have endings in the pleura, in which cardiovascular mechanical changes are at the most secondary stimuli, and it is unlikely that these particular fibers participate in the tonic reflex control of circulation.

Another important characteristic of sympathetic afferent fibers, which contributes to their widespread distribu-

tion, is the presence of branching fibers with more than one functional ending. The presence of sensory fibers with multiple endings was noted in some of the earliest anatomical descriptions of sympathetic nerves in the cat. Thus, Langley (1900) found that afferent sympathetic nerves from the thorax would occasionally branch in the periphery, and Ranson and Billingsley (1918a) when counting the large myelinated fibers (sensory) in the white rami and in peripheral nerve branches, came to the conclusion that sympathetic afferent nerves must divide into several smaller fibers shortly after leaving the white rami.

Sensory fibers with multiple terminals are common in the skin (Hunt and McIntyre, 1960), and have been described for sympathetic afferent fibers from the gut (Bessou and Perl, 1966; Morrison, 1973; Floyd and Morrison, 1974). In the first neurophysiological study of sympathetic afferent fibers from the thorax Holmes and Torrance (1959) described fibers with multiple terminals, but this property was more or less overlooked in many subsequent studies. Only recently (Coleridge et al., 1975; Malliani and Pagani, 1976) has the occurrence of fibers with multiple endings been described in more than a passing reference and the functional implications of this finding have been largely ignored.

Unless an investigator is deliberately searching for fibers with multiple terminals, it is easy to overlook them.

Towards the end of my series of experiments, when I was deliberately searching for fibers with multiple endings, I found that they accounted for fully 45% of the afferent fibers. Many of the multiterminal sympathetic afferent fibers had endings in the pleura that wraps around thoracic organs, and some fibers had several endings located along branching points of small blood vessels in the pleura and pericardium, a distribution that is characteristic of sympathetic afferent fibers from the gut (Bessou and Perl, 1966; Morrison, 1973; Floyd and Morrison, 1974) but has not earlier been described in the thorax. Others had endings clearly embedded in cardiovascular structures.

Neither in my experiments nor in those of Malliani and Pagani (1976) was there anything in the discharge pattern to distinguish fibers with multiple endings from those with only a single terminal. Malliani and Pagani (1976) suggested that fibers with multiple endings in the pleura are unlikely to provide the central nervous system with specific information, but that fibers with multiple vascular receptive fields may be particularly suited to signal generalized hemodynamic events. Nonetheless, they found no difference in the response to increased pressure of multiterminal fibers with all endings embedded in the wall of the thoracic aorta, and fibers with some endings in the aorta and others in the pleura.

RESPONSE TO INCREASED PRESSURE

The myelinated vagal baroreceptors from the heart and great vessels operate in the middle of their frequency range at normal blood pressure, with each heart beat they give a pulsatile discharge which is a frequency analogue of the pressure wave, respond accurately in a beat to beat manner to a decrease or increase in pressure, and they show very little adaptation to a prolonged change in pressure. Their transducer properties, therefore, appear to be particularly appropriate to participate in the tonic reflex control of the circulation. The mechanosensitive properties of sympathetic afferent fibers, on the other hand, are quite different, and they do not seem well adapted for a regulatory role. They operate at the low end of their frequency range at normal arterial pressure; a fall in pressure, such as produced by hemorrhage or shock, leads to only a small change in impulse activity (Malliani et al., 1973; Nishi et al., 1974; Lombardi et al., 1976); moreover, they give a small response to an increase in pressure, and their firing falls off rapidly when the pressure is maintained at a high level (Brown and Malliani, 1971; Nishi et al., 1974; Uchida, 1975a; Malliani and Pagani, 1976). Despite these very obvious differences in transducer function, Malliani and his colleagues in their numerous studies of sympathetic afferent fibers continue to suggest that they participate in the tonic reflex control of circulation (Malliani et

al., 1973; Malliani et al., 1975; Malliani and Pagani, 1976).

My observations on the response to pressure of afferent endings in the left ventricle, pulmonary veins and thoracic aorta are basically similar to those of Malliani and his colleagues (Brown and Malliani, 1971; Malliani et al., 1973; Malliani and Pagani, 1976). The fibers gave a small (10-15 imp/sec at the most) initial response to an increase in pressure, but the augmented discharge was only transient and firing fell off after a few (2-5) seconds even though pressure was maintained. Although there is no doubt that these fibers are mechanosensitive and that they signal a change in pressure, they are unlikely, because of their rapid adaptation, to provide the spinal cord with a continuous accurate error signal of deviations of blood pressure around the normal level. Thus, it seems unlikely that they represent a beat-to-beat cardiovascular regulatory system similar to the vagal and carotid sinus baroreceptors. Bessou and Perl (1966) found that sympathetic afferent fibers from the gut only gave a transient response to distension of the bowel, and they therefore called them "movement detectors". Similarly, the transducer properties of sympathetic afferent fibers from the heart and great vessels appear to be particularly suited to signal sudden changes in blood pressure, rather than participate in the tonic reflex control of the circulation.

Because sympathetic afferent fibers from the gut had a high sensitivity to outside touch, Bessou and Perl (1966) suggested that they may signal contact between loops of bowel. A similar hypothesis may be advanced for afferent fibers from the thorax; i.e. that they signal contact between thoracic viscera, although it is difficult to understand its functional implications.

In my sample, afferent fibers with endings embedded in the wall of the thoracic aorta were most sensitive to increases in pressure, and responded with an initial pulsatile discharge of 2-7 impulses per cardiac cycle, after which firing frequency diminished. Fibers with endings in the ventricular wall gave a smaller response (1 impulse/ heart beat) to similar changes in pressure (75-100 mmHg), but they did not adapt with maintained pressure. A similar difference between the responses of aortic and left ventricular endings is obvious in the published records of Malliani and coworkers (Malliani et al., 1973; Malliani and Pagani, 1976), although this difference is not discussed by these investigators. Other investigators (Brown and Malliani, 1971; Nishi et al., 1974) have found that afferent endings in more thin-walled structures such as the coronary arteries and pulmonary artery have qualitatively similar responses to those of the aorta described above. The responses of a given ending may possibly depend on factors such as the thickness and composition of the vessel wall,

and the orientation of the ending within it. It is likely that an ending located on the outer surface of a thick-walled structure like the left ventricle would be less affected by pulsatile changes in pressure than an ending in a thin-walled vessel like the pulmonary artery.

Although I found that endings in the vessel wall were most sensitive to changes in pressure, some endings in the pleura overlying the vessels gave a qualitatively similar response. Thus, mechanical changes in the heart and vessels are not only signalled by cardiovascular endings, but can also be signalled by endings from the associated connective tissue.

The secondary decrease in activity of sympathetic afferent fibers with a maintained increase in pressure has been referred to as "adaptation". Strictly speaking, however, the phenomenon of adaptation described by sensory neurophysiologists is the decrease in receptor discharge in the face of a constant stimulus (Mouncastle, 1974, p. 287). Arterial blood pressure causes a pulsatile stimulus, and one might expect the dynamic response of the ending to be renewed at each heart beat. In the case of sympathetic afferent fibers with endings in cardiovascular structures, however, the greatest increase in activity occurs in the first 4-5 cardiac cycles after pressure increases, and thereafter activity diminishes to a steady level that

rarely exceeds 1-2 imp/sec. Malliani and Pagani (1976) distended the thoracic aorta post mortem with a balloon, and found that endings in the aorta responded with a transient increase in impulse activity that was related to the change in pressure and rate of change in pressure. Thus, sympathetic afferent fibers "adapt" to a constant pressure stimulus as well as to a pulsatile pressure stimulus. The adaptation is most likely to be a function of the viscoelastic properties of the tissue surrounding the nerve endings. It may be significant that endings in a thick-walled structure like the left ventricle appear to have a more sustained activity than endings in the aorta, which suggests that adaptation is also a function of the thickness and composition of the innervated structure.

In conclusion, sympathetic afferent endings in the heart and great vessels are certainly mechanosensitive, for they are readily stimulated by probing. However, because they normally operate at the lower end of their frequency response, and because their response to an increase in pressure is transient, it is unlikely that they function as cardiovascular "baroreceptors" like the vagal endings in the heart and aorta. Their transducer properties appear to be more appropriate to signal sudden increases in blood pressure or venous return, such as may occur in situations that are threatening to the individual. In such situations a sudden increase in sympathetic afferent activity from the

heart and great vessels would be particularly valuable for the individual; it would possibly by positive feedback produce short term excitatory cardiovascular reflex effects - an increase in blood pressure, heart rate and myocardial contractility - such as described by Peterson and Brown (1971), Lioy et al. (1974) and Malliani et al. (1972), which would help the organism to mobilize in a "fight and flight" type reaction.

RESPONSE TO CHEMICAL AGENTS

There are other potentially dangerous circumstances in which sympathetic afferent input is engaged. Sympathetic afferent fibers from the heart signal cardiac pain (White et al., 1933; Lindgren and Olivecrona, 1947), and are stimulated by myocardial ischemia (Brown, 1967; Ueda et al., 1969; Brown and Malliani, 1971; Uchida and Murao, 1974d; Uchida and Murao, 1975). Although Malliani et al. (1973) found that small mylinated (Aδ) fibers from the heart were stimulated by experimental coronary occlusion only when the resultant myocardial ischemia caused abnormal distension of the cardiac wall, Uchida and Murao (1975) suggest that C-fibers, and possibly also Aδ fibers, are stimulated by chemicals released from the ischemic tissue. One of these chemicals, bradykinin, suggests itself as a very likely candidate. Bradykinin is one of the most potent naturally occurring pain-producing agents (Armstrong

et al., 1957; Elliott et al., 1960; Guzman et al., 1962; Lim et al., 1964; Sicuteri et al., 1966). The concentration of bradykinin increases to 0.2 μg/ml in coronary sinus blood during experimental coronary occlusion (Furukawa et al., 1969). Staszewska-Barczak et al. (1976) reported that topical application of bradykinin (0.02-5 μg in 1 ml saline) to the epicardial surface in lightly anesthetized dogs produced an excitatory nociceptive cardiac reflex whose afferent pathway was in the sympathetic nerve.

I observed reflex changes in blood pressure similar to those reported by Staszewska-Barczak et al. (1976) when I applied solutions of bradykinin to the surface of the heart in cats, and I examined the resultant changes in sympathetic afferent input. Two groups of investigators examined the effect of topical application of bradykinin on sympathetic afferent activity (Uchida and Murao, 1974a; Nishi et al., 1977), but obtained different results. For example, Uchida and Murao (1974a) found that endings in the heart responded with bursts of impulses for each cardiac cycle, indicating that bradykinin sensitized them to mechanical events in the heart. Nishi et al. (1977), on the other hand, found that topical application of a filter paper soaked in 80 μg/ml bradykinin (a very high concentration compared to that found in the ischemic heart) to endings on the left ventricle produced bursts of impulses even after the heart had stopped, suggesting direct chemical excitation

of the endings. In both these studies, moreover, there is little quantitative information about the effect of bradykinin. When I applied bradykinin (1 μg/ml), a concentration which produced a nociceptive pressor reflex when applied to the epicardial surface in lightly anesthetized dogs (Staszewska-Barczak et al., 1976), 90% of endings were stimulated, ventricular and atrial endings being particularly sensitive to bradykinin.

There were three major patterns of response to bradykinin. Some endings responded with a cardiac pattern, i.e. with impulses always occurring at the same phase of the cardiac cycle. Since epicardial application of bradykinin often produced a pressor response in my experiments, one cannot discount the possibility that in some experiments the response was secondary to mechanical changes in the heart and great vessels, such as increased cardiac contraction or increased blood pressure. However, on the average, the response of cardiac endings occurred with a shorter latency (12.2 sec for ventricular endings; 9.2 sec for left atrial endings) than did the pressor response (16.6 sec). Moreover, some endings responded with a cardiac discharge in the absence of a reflex increase in pressure. Thus, the response of these endings to bradykinin may well have been due to a sensitization to mechanical events as suggested by Uchida and Murao (1974a).

Other endings responded with a low-frequency discharge that had no regular relation to the cardiac cycle, so that they were probably stimulated by bradykinin rather than sensitized.

Finally, 21 of the endings, many of them on the heart, fired with bursts of 9-25 imp/sec after topical application of 1 μg/ml bradykinin. These were by far the most chemically sensitive fibers, and there seemed to be no doubt that the afferents were stimulated directly by bradykinin. I think it is primarily these endings that represent the afferent arm of the nociceptive pressor reflex evoked by epicardial application of bradykinin. These endings may have been particularly sensitive to chemical agents, or they may have been most accessible to bradykinin because of their position on the epicardial surface. Earlier studies, particularly by the Japanese investigators, suggest that there is a subgroup of sympathetic afferent endings supplied by C-fibers that are less sensitive to mechanical stimuli but are very sensitive to chemical agents (Uchida et al., 1969; Takenaka et al., 1970; Brown and Malliani, 1971; Nishi and Takenaka, 1973; Uchida and Murao, 1974c). My results can neither support nor refute this hypothesis, because the number of C-fibers in my sample was too small to draw any definite conclusions. However, all but one of the endings that gave the greatest response to bradykinin had a conduction velocity of 6 m/sec

or less. Moreover, some of the endings that were very sensitive to bradykinin application were difficult or impossible to stimulate by probing the region of the terminals. It is conceivable, therefore, that my method of selection left substantially unexplored a population of small fibers whose endings respond primarily to chemical stimuli and very little if at all to mechanical stimuli.

When I applied bradykinin repeatedly to an afferent ending the response became progressively smaller. This phenomenon of tachyphylaxis or desensitization was displayed by all endings, and was independent of their location and their pattern of response to bradykinin. Tachyphylaxis seemed to be a property of the endings themselves and not of the fiber, because when one ending of a multiterminal afferent fiber was desensitized by repeated application of bradykinin, a high frequency discharge could then be evoked by applying bradykinin to a second ending of the same afferent fiber. Surprisingly, the occurrence of tachyphylaxis with repeated application of bradykinin was not reported by Uchida and Murao (1974a). However, tachyphylaxis may well have accounted for the apparent reduction in response to bradykinin after injection of acetyl salicylic acid. It is also surprising that Staszewska-Barczak et al. (1976) did not report tachyphylaxis when investigating the excitatory nociceptive pressor reflex evoked by repeated epicardial application of bradykinin in dogs.

Examination of their records, however, show that their pressor response was quite small (maximum 15 mmHg). It seems very likely that tachyphylaxis was already a feature of this response, and that the potentiation attributed to application of PGE_1 was merely a reversal of this phenomena.

The development of tachyphylaxis with repeated application of bradykinin has been observed in studies of a variety of sensory nerves. Elliott et al. (1960) found that application of bradykinin to a blisterbase in the skin in man produced pain, but with repeated applications the pain diminished. Beck and Handwerker (1974) and Handwerker (1976) recorded impulse activity from myelinated and unmyelinated cutaneous afferent fibers in the cat. The fibers were stimulated by i.a. injection of bradykinin, but with repeated application at 1-15 minute intervals the response decreased. Tachyphylaxis has also been described for the pseudoaffective pain-response to intraarterial injection of bradykinin in the spleen in dogs (Hirata et al., 1966), and for the bradykinin-evoked bronchoconstriction in guinea pigs (Elliott et al., 1960).

Little is known about the cellular mechanism that causes the response to bradykinin to decrease with repeated applications. However, studies on other agents, such as acetylcholine, catecholamines, insulin and a number of peptide hormones have revealed several cellular mechanisms

by which tachyphylaxis may occur. For example, when the nicotinic receptor is exposed to acetylcholine, there is an increase in the affinity between receptor and agonist, so that "old" acetylcholine molecules become tightly bound to the receptors and thereby prevent binding of newly applied molecules (Changeaux et al., 1975). A different mechanism has been proposed for the desensitization of β-adrenergic receptors in frog erythrocytes. According to Lefkowitz et al. (1978), the presence of catecholamines does not change the affinity between receptor and agonist, but it induces a conformational change in the receptors so that they can no longer bind the catecholamines. In the case of several hormones (insulin, calcitonin, growth hormone, TRH), it has also been shown that binding of the ligand to the receptor may lead to a reduction of the number of receptors in the cell membrane (see Kahn, 1976). This may occur by enzymatic degradation of the receptor, by shedding of the receptor-hormone complex into the medium, or by reuptake (endocytosis) of the receptor-hormone complex into the cell, as has been recently demonstrated for insulin and epidermal growth factor (Schlessinger et al., 1978). Finally, recent evidence suggests that tachyphylaxis to peptide hormones may be due to changes distal to the receptors which changes the cellular level of cyclic AMP. For example, it may be due to production of nonspecific antagonists for adenyl cyclase activity, cellular extrusion of cyclic AMP, or increased

degradation of cyclic AMP by phosphodiesterase (see Insel, 1978). Although little is known about tachyphylaxis to bradykinin, its cellular mechanism may be similar to that described for the other agents.

The algesic effects of bradykinin seem to be closely associated with tissue levels of prostaglandins. It was early discovered that the algesic effects of intraarterial and intraperitoneal injection of bradykinin could be reduced or abolished by aspirin-like drugs (Coffman, 1964; Lim et al., 1964; Sicuteri et al., 1966; Lim et al., 1967; Collier et al., 1968). Vane and coworkers subsequently showed that aspirin-like drugs inhibit the biosynthesis of prostaglandins in such different experimental systems as cell free homogenate of guinea pig lungs (Vane, 1971), in the isolated, perfused dog spleen (Ferreira et al., 1971) and in human platelets (Smith and Willis, 1971). Thus, many of the effects of bradykinin seemed to be explained by activation of the prostaglandin generating system. Like bradykinin, prostaglandin E's are released from the ischemic heart, and Berger et al. (1976) found that the concentration of prostaglandins in coronary sinus blood increased from 0.44 to 1.07 ng/ml during experimental coronary occlusion.

Prostaglandins themselves can cause pain when infused over a long period of time, or when applied in high (i.e. mg) concentrations. For example, after prolonged (several

hours) infusion of prostaglandins (5-10 μg/min) into superficial veins of the hand and forearm in man, a burning pain developed at and above the infusion site (Karim, 1971; Collier et al., 1972; Gillespie, 1972). Moreover, Collier and Schneider (1972) reported that intraperitoneal injection of 0.1-1 mg/kg prostaglandins in mice induced a pain-like writhing response. On the other hand, PGE_1 did not cause pain when solutions of 0.1-100 μg in 1 ml saline were applied to a blisterbase in the skin in man (Horton, 1963), or when 25-100 ng prostaglandins were injected intradermally in man (Crunkhorn and Willis, 1971). Ferreira (1972) subsequently showed that in concentrations likely to be present in inflammatory reactions (ng levels), the effect of intradermal injection of prostaglandins seemed to be that of long lasting hyperalgesia, i.e. sensitization of the pain receptor to mechanical or chemical stimuli.

My observations regarding the effect of PGE_1 are in keeping with the results of Ferreira (1972) and Staszewska-Barczak et al. (1976). Staszewska-Barczak reported that application of PGE_1, E_2 and $F_{2\alpha}$ (0.01-0.1 μg/ml) to the epicardial surface in lightly anesthetized dogs produced no appreciable cardiovascular changes, and similarly I found that topical application of 0.1 μg/ml PGE_1, and also 100 times this concentration (10 μg/ml), to a large sample of sympathetic afferent fibers only stimulated 13% of the fibers tested. The endings of both Aδ and C-fibers were

stimulated by PGE_1; some fired with high frequency bursts, others responded with a low frequency discharge and with either a cardiac rhythm, or with impulses independent of cardiac events. Almost all the endings stimulated by PGE_1 were on the heart.

Staszewska-Barczak et al. (1976) found that epicardial application of prostaglandins, in a concentration which by itself produced no reflex cardiovascular changes, increased the pressor responses induced by epicardial application of bradykinin. Although this effect occurred in dogs in which endogenous prostaglandin synthesis was unimpaired, it was particularly striking in dogs given indomethacin. I examined the interaction of bradykinin and PGE_1 on the afferent fibers which produce these reflex effects. Except for minor differences, my results are in general agreement with the observations of Staszewska-Barczak et al. (1976), moreover they provide information about the mechanism of interaction between bradykinin and PGE_1.

In initial experiments, I found that the intensity of stimulation produced by combined application of bradykinin and PGE_1 differed little from that produced by application of bradykinin alone. Thus, PGE_1 did not appear to potentiate the response to bradykinin, as suggested by Staszewska-Barczak et al. (1976). However, these early experiments were done before I realized the full significance of the

phenomenon of tachyphylaxis.

In subsequent experiments I followed another protocol used by Staszewska-Barczak et al. (1976), and examined the response to bradykinin after infusing indomethacin, and after applying PGE_1 to the receptive site for 20 minutes. The changes in impulse activity were in the same direction as the reflex changes in blood pressure reported by Staszewska-Barczak et al. (1976). Thus, PGE_1 (0.1 μg/ml) alone had no effect on impulse activity but it did increase the response to bradykinin.

It gradually became obvious that I would progress no further in elucidating the mechanism of interaction of bradykinin and PGE_1 unless I used a protocol that took the phenomenon of tachyphlaxis into account. Using such a protocol (applying bradykinin repeatedly to the afferent endings after giving indomethacin and after applying PGE_1), I found that PGE_1 restored the afferent response to bradykinin and abolished or reduced tachyphylaxis. It is clear that the increased responsiveness to bradykinin after administration of PGE_1 was not due to the longer interval between the two bradykinin applications (i.e. 20 minutes instead of 15 minutes). In the course of my experiments on sympathetic afferent fibers I usually observed tachyphylaxis in the response to repeated application of bradykinin at 20-minute intervals. Moreover, the second response to brady-

kinin after PGE_1 was no less than the first. In addition, in control experiments on three endings the response to bradykinin was decreased after 0.9% NaCl-solution had been applied to the receptor site for 20 minutes, but in all of these endings the response to bradykinin was then significantly increased after PGE_1 had been applied to the endings for 20 minutes.

Results obtained from the last potentiation protocol suggest that PGE_1 acted by counteracting the tachyphylaxis to bradykinin. In retrospect, I now see that this was also demonstrated in the first potentiation experiments. Whereas I found that repeated applications of bradykinin alone to sympathetic afferent nerve endings always produced tachyphylaxis, in the first potentiation experiments I found that the intensity of stimulation produced by application of bradykinin and PGE_1 together was not different from that produced by the earlier application of bradykinin. In other words, when PGE_1 was given together with the second application of bradykinin, tachyphylaxis did not occur.

Handwerker (1976) has described a similar interaction between bradykinin and PGE_1 on single cutaneous C-fibers in the cat: Injection of bradykinin (5 µg in 0.5 ml tyrode solution) into the femoral artery evoked bursts of impulses in the afferent fiber. With repeated injections at 1-minute

interval the response diminished, but after continuous infusion of 5 μg PGE_1/min the response to bradykinin was restored and tachyphlaxis abolished.

What are the mechanisms by which PGE_1 potentiates the response to bradykinin and keeps the nerve endings sensitive to repeated applications of bradykinin? Since Ferreira et al. (1973) found that other vasodilators, like eledoisin and nitroglycerin did not potentiate the nociceptive response to bradykinin in the dog spleen whereas prostaglandins did, it seems unlikely that PGE_1 exerts its potentiating effect by increasing the vascular permeability to bradykinin. Several other theories have been suggested to explain the potentiating action of PGE_1. Pickles et al. (1966) found that prostaglandins (E and F) potentiated the effect of other spasmogens on rat and guinea pig myometrium, and they hypothesized that PG's produced this effect by combining with specific receptors, or at specialized "pores" in the membrane, and that prostandin-molecules were used repeatedly as membrane carriers.

PGE_1 affects the cellular concentration of Ca^{++} in several ways. For example, Coceani et al. (1969) found that PGE_1 caused retention of CA^{++} in rat stomach strips and accumulated Ca^{++} in a slowly exchangeable pool, and Kirtland and Baum (1972) reported that PGE_1 acts as a "calcium ionophore" and facilitates binding of Ca^{++} to the

inner mitochondrial membrane. In an attempt to explain the contracting effect of PGE_1 on the isolated rabbit aorta, Strong and Bohr (1967) suggested that PGE_1 decreased the binding of ionic CA^{++} at fixed sites in the cell membrane, a change which would decrease the membrane stability (Rasmussen, 1970). It is possible that a similar mechanism accounts for the interaction of PGE_1 and bradykinin on sympathetic afferent nerve endings.

Bradykinin and PGE_1 may also interact through action on the same intracellular agent(s), such as cyclic AMP. Bradykinin binds to receptors on the cell membrane (Rocha e Silva, 1970). Although little is known about its mechanism of action, since bradykinin is a peptide, it is possible that it exerts its effect through activation of the adenyl cyclase/cyclic AMP system. Prostaglandins also bind to receptors on the cell membrane, and PGE_1 increases the cellular level of cyclic AMP (Butcher and Baird, 1968; Kuehl et al., 1973). It has recently been shown that tachyphylaxis to peptide hormones may be due to activity changes in enzymes involved in the production or breakdown of cyclic AMP (see Insel, 1978). Since PGE_1 potentiates the sympathetic afferent response to bradykinin by diminishing tachyphylaxis, it is possible that the interaction of bradykinin and PGE_1 on the endings involves the adenyl cyclase/cyclic AMP system.

This study of chemical excitation of sympathetic afferent nerve endings has important clinical implications. Sympathetic afferent nerve fibers from the heart signal cardiac pain (White et al., 1933; Lindgren and Olivecrona, 1947). Bradykinin is a potent algesic agent which is released from the ischemic heart (Furukawa et al., 1969; Kimura et al., 1973), and as shown by Uchida and Murao (1974a), Nishi et al., (1977) and also in this study, bradykinin stimulates sympathetic afferent endings on the heart. Prostaglandins are also released from the ischemic heart (Berger et al., 1976), and although PGE_1 only stimulates a few sympathetic afferent nerve fibers, prolonged application of PGE_1 potentiates the response to bradykinin and abolishes tachyphylaxis. Thus, when bradykinin is released during myocardial ischemia, it will stimulate sympathetic afferent nerve endings in the heart and produce cardiac pain with concomitant cardiovascular pressor reflexes (Staszewska-Barczak et al., 1976). The pain will be further increased by simultaneous release of prostaglandins from the ischemic heart, which will potentiate the effect of bradykinin on the nerve endings by maintaining their sensitivity to bradykinin. Although I have only examined the effect of two endogenous agents, bradykinin and PGE_1, on sympathetic afferent nerve endings, a number of other endogenous agents, such as lactic acid, adenosine and its phosphorylated intermediates and K^+, are released

from the ischemic heart (Conn et al., 1959; Imai et al., 1962; Haddy and Scott, 1971). All of these agents stimulate sympathetic afferent nerve endings (Uchida et al., 1969; Uchida and Murao, 1974b and c; Uchida and Murao, 1975), and although the interaction of these agents on sympathetic afferent nerve endings has not been examined, other experiments have shown that several of these substances may interact on pain receptors (Sicuteri et al., 1966; Juan and Lembeck, 1974). It is probably the interaction of all these chemical agents on sympathetic afferent nerve endings from the heart during myocardial ischemia which produces the full fledged clinical picture of cardiac pain.

NERDRUM, TONE (Ph.D., Physiology)

SYMPATHETIC AFFERENT IMPULSES FROM THE HEART, GREAT VESSELS, PERICARDIUM AND PLEURA OF THE CAT: THE EFFECT OF MECHANICAL AND CHEMICAL STIMULI

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Abstract of a doctoral dissertation at the University of California, San Francisco

Thesis supervised by Professor John C. G. Coleridge

Since the end of the nineteenth century the sympathetic afferent input has been known to include a system of nociceptors that signal visceral pain (White, 1957). During the past decade, the idea has developed that these fibers function as mechanoreceptors that provide tonic information about mechanical events in the heart and great vessels (Malliani et al., 1975). The aim of this thesis was to investigate the effect of mechanical and chemical stimuli on sympathetic afferent fibers from the heart, great vessels, pericardium and pleura, to obtain some insight into the relative importance of the two modes of stimulation.

I recorded impulse activity from the upper left sympathetic chain and white rami in cats. The fibers had a low

resting discharge and less than half had a cardiac rhythm; the majority fired irregularly or were silent at normal arterial pressure.

The endings were extremely sensitive to touch, which made them easy to locate. They were distributed throughout the left side of the thorax, and although I found many in the wall of cardiovascular structures, many were in the overlying pericardium and pleura. Many fibers had multiple (2-6) discrete (often widely separated) endings. In some cases, the endings of a single parent fiber were distributed to a single thoracic structure (e.g. pleura, aorta), in others the endings of a single fiber were distributed to several structures.

In contrast to their high frequency evoked by probing (40-100 imp/sec), cardiac or aortic endings were remarkably insensitive to large (75-100 mmHg) increases in pressure. Aortic endings responded with an initial pulsatile discharge of 2-7 imp/cardiac cycle, but impulse activity quickly declined to 1 impulse/cardiac cycle or less even though pressure was maintained. Left ventricular fibers appeared less sensitive to changes in pressure than aortic fibers. Because of their relatively small and often transient response to increased pressure, it seems unlikely that sympathetic afferent fibers from the heart and great vessels function as cardiovascular baroreceptors like those in

the aortic and carotid sinus nerves.

Recent evidence suggests that metabolites released during myocardial ischemia stimulate sympathetic afferent endings in the heart and produce cardiac pain. Both bradykinin (BK) and prostaglandin E_1 (PGE_1) accumulate in the ischemic heart (Kimura et al., 1973; Berger et al., 1976). Staszewska-Barczak et al. (1976) found that application of BK to the ventricular surface in lightly anesthetized dogs produced a nociceptive pressor reflex that was potentiated by PGE_1 and abolished by sympathectomy. I examined the afferent arm of this nociceptive reflex, and I first examined the effects of BK and PGE_1 applied separately to sympathetic afferent endings. Topical application of BK (1 μg/ml) stimulated 89.8% of all endings. Although most endings responded with 4-5 imp/sec, about 20% of the endings were extremely sensitive and fired with high frequency discharge of 9-25 imp/sec. All endings showed tachyphylaxis to repeated application of BK. PGE_1 (0.1-10 μg/ml) alone stimulated only a few endings (most of which were on the heart), but it potentiated the response to BK. I applied BK (1 μg/ml) repeatedly at 15-minute intervals after infusing indomethacin (5 mg/kg), and for each application the response became progressively smaller. I then applied PGE_1 (0.1 μg/ml, 0.3-0.5 ml/min) for 20 minutes. PGE_1 alone had no significant effect on firing, but subsequent reapplication of BK evoked an increase in impulse activity which was

significantly greater than that evoked before treatment with PGE_1. When I continued the PGE_1-treatment and reapplied BK after 15 minutes, there was very little tachyphylaxis. In conclusion, pain and pressor reflexes in myocardial ischemia can result from stimulation of afferent sympathetic fibers by BK, and the sensitivity of nerve endings is maintained at a high level by simultaneous release of PGE_1.

APPENDIX I

ANATOMY OF SYMPATHETIC NERVES TO THE THORACIC VISCERA IN THE CAT

ANATOMY OF SYMPATHETIC NERVES TO THE THORACIC VISCERA IN THE CAT

In the cat, the stellate ganglion is a large compound ganglion formed by the fusion of inferior cervical ganglion and the upper three (and sometimes four) thoracic ganglia (Ranson and Billingsley, 1918a). It is located at the angle of rib 2, and is connected to the middle cervical ganglion by the two limbs of ansa subclavia, which loop around the subclavian artery. Distal to the stellate ganglion, the sympathetic ganglia are segmentally arranged (see Fig. 28).

The stellate ganglion receives white and grey rami from the first three thoracic spinal nerves, and sometimes also from the fourth. The white and grey rami of the upper two or three thoracic nerves are usually fused. The first two of these mixed rami run directly to the stellate ganglion in the cat, whereas the third joins the trunk a short distance below and ascends in a common sheath with the trunk to reach the ganglion. In the experiments, I was unable to distinguish morphologically between white and grey filaments in these mixed rami, and when filaments were placed on the recording electrodes, they often contained

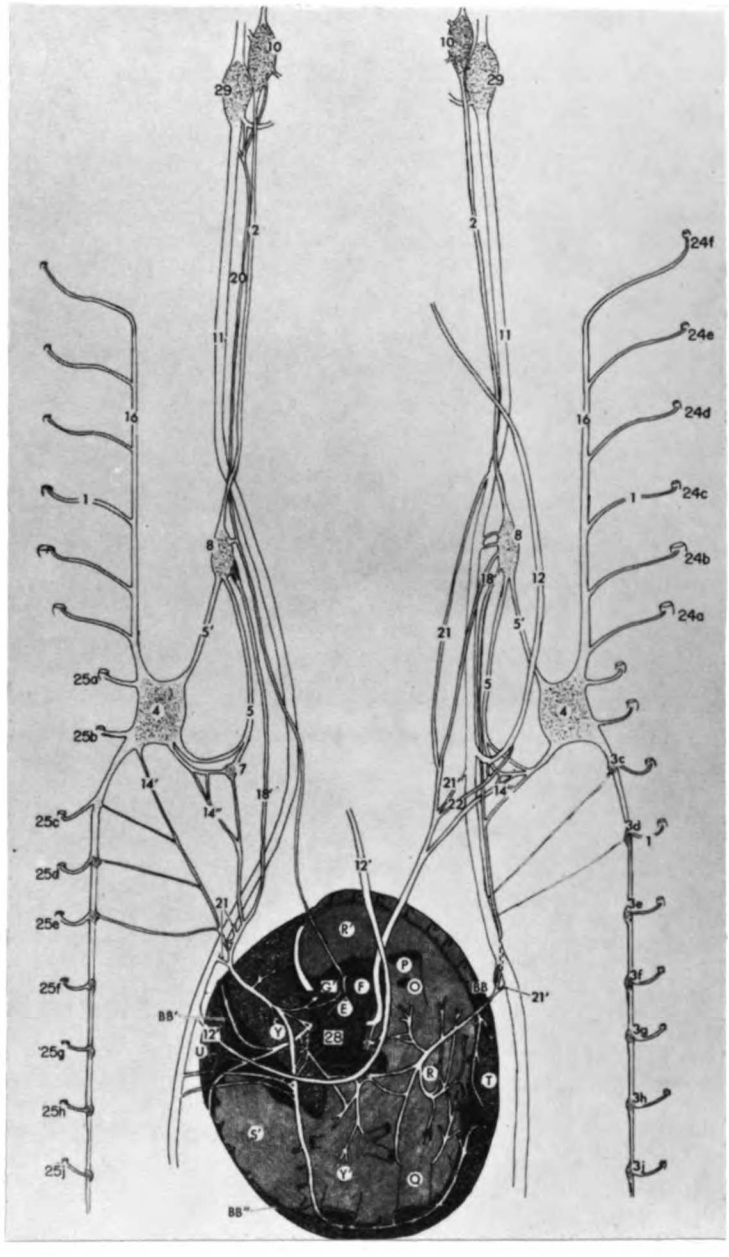

Fig. 28. Dorsal view of the cardiac innervation in the cat. The sympathetic trunks and vagi are reflected laterally. From: McKibben, J. C. and Getty, R. (1968). A comparative morphologic study of the cardiac innervation in domestic animals. II The feline. Am. J. Anat., 122, 545-554.

ABBREVIATIONS

1	Ramus communicans
2	Sympathetic trunk
3c	Third thoracic ganglion
3d	Fourth thoracic ganglion
3e	Fifth thoracic ganglion
3f	Sixth thoracic ganglion
3g	Seventh thoracic ganglion
3h	Eighth thoracic ganglion
3j	Ninth thoracic ganglion
4	Stellate ganglion
5	Ansa subclavia (ventral limb)
5'	Ansa subclavia (dorsal limb)
7	Intermediate ganglion
8	Middle cervical ganglion
10	Superior cervical ganglion
11	Vagus nerve
12	Right recurrent laryngeal nerve
12'	Left recurrent laryngeal nerve
13	Thoracic cardiac nerve
14'	Inferior cardiac nerve
14"	Middle cardiac nerve
16	Vertebral nerve
18'	Superior cardiac nerve
20	Cranial cervical nerve
21	Cranial vagal cardiac nerve
21'	Caudal vagal cardiac nerve
22	Recurrent cardiac nerve
24a	Eighth cervical spinal nerve
24b	Seventh cervical spinal nerve
24c	Sixth cervical spinal nerve
24d	Fifth cervical spinal nerve
24e	Fourth cervical spinal nerve
24f	Third cervical spinal nerve
25a	First thoracic spinal nerve
25b	Second thoracic spinal nerve
25c	Third thoracic spinal nerve
25d	Fourth thoracic spinal nerve
25e	Fifth thoracic spinal nerve
25f	Sixth thoracic spinal nerve
25g	Seventh thoracic spinal nerve
25h	Eighth thoracic spinal nerve
25j	Ninth thoracic spinal nerve
26	Vascular nerve
28	Cardiac plexus
29	Nodose ganglion
E	Aorta
F	Brachiocephalic artery
G'	Left subclavian artery
O	Right azygos vein
P	Superior vena cava
Q	Inferior vena cava
R	Right atrium
R'	Right auricle
S'	Left auricle
T	Right ventricle
U	Left ventricle
Y	Pulmonary artery
Y'	Pulmonary vein
BB	Right coronary artery
BB'	Descending branch of left coronary artery
BB"	Circumflex branch of left coronary artery

efferent as well as afferent impulse activity. From the fourth to the eighth thoracic spinal nerves the white and grey rami are separate and easy to distinguish from one another, and they join the trunk at the level of the corresponding ganglion.

Sympathetic fibers reach the viscera through various visceral nerves. The heart is innervated by the superior, middle and inferior cardiac nerves, as well as by the thoracic cardiac nerves. In the cat, the superior cardiac nerve originates as 3-4 filaments from the inferior pole of the middle cervical ganglion, the middle cardiac nerve arises from the ventral limb of the ansa subclavia and the inferior cardiac nerve usually arises as 3-4 nerve filaments from the stellate ganglion (McKibben and Getty, 1968). The thoracic cardiac nerves originate from the sympathetic ganglia and interconnecting chain caudal to the stellate ganglion. These nerves have been discovered and rediscovered throughout history, but were first described by Swan in 1830. The extent of these nerves in the cat has been somewhat disputed. Saccomanno (1943) observed at least 10 thoracic sympathetic nerves on either side from the upper eight thoracic segments, and when he examined the nerves histologically, he found many additional nerves that were too small to be observed macroscopically. On the other hand, McKibben and Getty (1968) found only a few thoracic sympathetic nerves on the left side, arising from the third

to fifth thoracic ganglia, and none on the right.

The ascending aorta and aortic arch receive sympathetic nerve-fibers from the stellate ganglion and upper thoracic ganglia, and most fibers follow the thoracic cardiac nerves. The descending aorta is supplied by direct fibers from the ganglia caudal to the stellate ganglion.

In the cat, pulmonary artery and veins are innervated by the middle cardiac nerve on the left side and the inferior cardiac nerve on the right (McKibben and Getty, 1968). They are also innervated by nerve fibers from the second to fifth or sixth thoracic ganglia.

Because I recorded impulse activity from the left sympathetic chain and white rami, I never recorded activity from fibers with endings in the thoracic veins. In the cat, the superior and inferior vena cava are supplied by the right inferior cardiac nerve, whereas the azygos and hemiazygos veins are innervated by right thoracic cardiac nerves (McKibben and Getty, 1968).

The sympathetic nerves go to the respective nerve plexuses (the cardiac, pulmonary or aortic plexuses), where they intermingle with vagal branches. The cardiac plexus consists of a superficial portion located in the concavity of the aortic arch, and a deeper portion between the arch and the tracheal bifurcation. It is subdivided into right

and left halves, which supply the right and left coronary plexuses, respectively. The pulmonary plexus is located on the posterior aspect of the lung root, and the aortic plexus is in the walls of the thoracic aorta.

The afferent sympathetic fibers pass through the white rami, and they consist of unmyelinated fibers, small myelinated fibers and a few large myelinated fibers of diameter 5-13 μ (Ranson and Billingsley, 1918a). In addition, there is evidence that a few afferent fibers pass through the grey rami (Langley, 1896).

Sympathetic afferent fibers have their cell bodies in the dorsal root ganglion. This was shown by Langley, and Ranson and Billingsley. No degeneration was seen in the white rami after section of the sympathetic or splanchic nerves, but if the spinal nerves were sectioned just peripheral to the dorsal root ganglion, all but a few of the myelinated fibers in the white rami degenerated (Langley, 1903). However, after section of the ventral roots, which eliminated preganglionic sympathetic efferent fibers, the white rami contained unmyelinated fibers and myelinated fibers of all fiber sizes (Ranson and Billingsley, 1918a and b).

The sympathetic nerves contain both afferent and efferent nerve fibers. Saccomanno (1943) analyzed the fibercomposition of the thoracic cardiac nerves in the cat.

In degeneration studies in which he sectioned the dorsal roots distal to the spinal ganglion on one side and used the other side as a control, he estimated that the majority of the sensory nerves, or ca. 70%, were C-fibers and that only 30% were small myelinated fibers.

From the respective plexuses (cardiac, pulmonary and aortic), mixed sympathetic and vagal nerves are distributed to the viscera. Many of the large myelinated fibers that supply the heart are probably sympathetic. Nettleship (1936) found that many of them degenerated when he excised the dorsal root ganglia of the upper thoracic spinal nerves, but that relatively few degenerated after vagal section distal to the nodose ganglion.

Many nerve endings have been described in the walls of the heart, and they have all been of the unencapsulated type, like free nerve endings or dot-like "beaded" endings or various degrees of complexity (Nettleship, 1936). Although many of these endings undoubtedly belong to vagal and sympathetic efferent fibers and to vagal afferent fibers, some endings must also belong to sympathetic afferent fibers that terminate in the heart.

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