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The site of action of clonidine on the endocrine and cardiovascular systems

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THE SITE OF ACTION OF CLONIDINE ON THE ENDOCRINE AND CARDIOVASCULAR SYSTEMS

by

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DISSERTATION

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ABSTRACT

The Site of Action of Clonidine

on the Endocrine and Cardiovascular Systems

The pharmacological agent, clonidine, acts in the central nervous system to alter various endocrine and cardiovascular parameters. These experiments were performed in order to determine where clonidine exerts these effects in the central nervous system.

A method of catheterizing the vertebral and carotid arteries in the dog is described. The regional distribution to the brain of these arteries is studied by infusing radionuclide labelled microspheres. The vertebral arteries primarily perfuse the hindbrain, hypothalamus and posterior cortical regions. The carotid arteries primarily perfuse the rostral portions of the brain including the hypothalamus and posterior coritcal region. In dogs with the basilar artery clipped at the mid-pontine level the vertebral arteries perfuse the region caudal to the clip and the carotid arteries perfuse the rest of the brain.

Clonidine was infused into the carotid and vertebral arteries of dogs with and without the basilar artery clipped. These studies demonstrate that clonidine acts in the medulla or pons to decrease blood pressure. The results do not demonstrate that at the doses used clonidine's effects on heart rate are centrally mediated. Plasma renin activity is decreased by clonidine's action at a region in the brain which probably is located in the midbrain, hypothalamus or posterior cortical region. Clonidine decreases plasma ACTH levels and increases plasma GH levels by acting at a site in rostral portions of the brain. It may affect ACTH and GH secretion by actions in the hypothalamus or posterior cortical region.

Preface

The experiments described in this dissertation were performed under the auspices of Dr. William F. Ganong. In experiments on the relationship between the central nervous system and the endocrine system he and other investigators have demonstrated that the pharmacological agent, clonidine, acts in the central nervous system to alter various endocrine and cardiovascular parameters. These experiments were performed in order to determine where clonidine exerts these effects in the central nervous system.

The first section of this dissertation describes the previous work on the physiology and pharmacology of clonidine action. This section includes discussions of the possible mechanisms by which clonidine exerts it effects, and also includes a discussion of the likely sites of action of clonidine. Since the initial work on the pharmacology of clonidine was primarily concerned with its actions on the cardiovascular system, the discussion initially focuses on these aspects of its action. This is followed by a discussion of the more recent work on clonidine's endocrine effects. The end of this section explains the rationale for the experiments which were performed for this dissertation.

The second section describes and validates the methods used in these studies. The final section describes the experimental results and then discusses the meaning of these results in light of the other experimental evidence available.

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Section I

INTRODUCTION

Some imidazoline compounds are potent stimulators of alpha-adrenergic receptors (Mujic and van Rossun, 1965) and therefore are good nasal decongestant agents. In search of a better decongestant, Dr. Stahle, a chemist at Boehringer-Ingelheim, synthesized various imidazoline derivatives and routinely submitted these agents for evaluation by the company's medical department. A physician in this group, Dr. Wolf, administered about a milligram of one of these agents, called clonidine, to himself and discovered it had sedative, bradycardic and hypotensive effects (Graubner and Wolf, 1966). Although many other actions of clonidine have been described since these initial observations, the depressant actions of clonidine on the cardiovascular system have stimulated the most research and investigation. Therefore most of the research on clonidine's molecular or receptor mechanism of action has been directed towards understanding these effects.

The first animal studies on the action of clonidine (Hoefke and Kobinger, 1966) showed that when clonidine (10-30%) was injected intravenously as a bolus there was an initial hypertensive response followed by hypotension. When clonidine was slowly administered intravenously (Nayler et al, 1968) or given orally (Onesti et al, 1969) only the hypotensive response was seen. The blood pressure response to clonidine may also depend on the resting blood pressure. By using various anesthetic agents which effect the cardiovascular system in different ways, Kündig et al (1967) demonstrated that if the initial

blood pressure was high, intravenous administration of clonidine would decrease blood pressure; however, when the initial blood pressure was low the same dose of clonidine would have a hypertensive effect. These differences could be due to some other action of the anesthetics.

I.A. The Hypertensive Action of Clonidine

Further pharmacological studies have demonstrated the the hypertensive action of clonidine results from the constriction of peripheral blood vessels. Several investigators have demonstrated that phentolamine, an alpha-receptor blocking drug, blocks the hypertensive response to clonidine (Kobinger and Walland, 1967a; Rand and Wilson, 1968; Boissier et al, 1968). Experiments using perfused skeletal muscle preparations (Nayler et al, 1966; Constantine and McShane, 1968) demonstrated that clonidine increased vascular resistance. This action was blocked by phentolamine and phenoxybenzamine.

There are several possible explanations of these results. Clonidine could directly stimulate alpha-adrenergic receptors or could cause the release of endogenous norepinephrine and thus elicit its effects in a manner similar to tyramine. Since clonidine causes constriction of isolated arteries after sympathectomy (Robinson et al, 1967; Constantine and McShane, 1968; Boissier et al, 1968) the latter possibility seems unlikely. It is also unlikely that clonidine exerts its effects by blocking reuptake of released transmitter (which would increase synaptic cleft concentrations of transmitter) since it causes marked vasoconstriction in the presence of cocaine, a reuptake blocker (Robinson et al, 1967).

Other classical responses to alpha-adrenergic stimulation including contraction of the nictitating membrane of the cat (Rand and Wilson, 1968) and constriction of isolated seminal vesicles (Boissier et al, 1968) are also elicited by clonidine. Phentolamine blocks these actions. Thus, several lines of evidence indicate that clonidine does stimulate alpha-adrenergic receptors in peripheral tissues and that classical alpha-blocking drugs can prevent clonidine from exerting its effects on these receptors.

Another action which may contribute to the hypertensive effect of clonidine is its inotropic action. This action may be due to the ability of clonidine to stimulate H_2 receptors (Karppanen and Westermann, 1973). In isolated heart preparations the inotropic effect of clonidine was antagonized by burimamide, an H_2 blocking drug (Csongrady and Kobinger, 1974).

I.B. The Blood Pressure and Heart Rate Lowering Actions of Clonidine

The ability of clonidine to decrease blood pressure is not as simple to explain as its hypertensive effect. However, it is obvious that clonidine has multiple actions on the cardiovascular system since heart rate declines in spite of the decrease in blood pressure when clonidine is administered to animals. The possible therapeutic effects of an agent with these properties in the treatment of hypertension has stimulated a great deal of research into how the effects are mediated (Onesti et al, 1969; Ng et al, 1967; Hoobler and Sagastume, 1971).

Several possible mechanisms by which clonidine might lower blood pressure have been explored. Since the cardiovascular changes induced by clonidine are similar to the changes caused by ganglion blockade (Kobinger and Walland, 1967b), it initially seemed likely that clonidine blocked transmission in sympathetic ganglia. However, in experiments in which pre-ganglionic nerves were stimulated, clonidine had no effect on transmission in autonomic ganglia (Hoefke and Kobinger, 1966; Rand and Wilson, 1968; Boissier et al, 1968; Dhasmana et al, 1972).

Another possible explanation of how clonidine decreases blood pressure and heart rate is that it interferes with transmission at the synapses of post-ganglionic sympathetic fibers. Constantine and McShane (1968) demonstrated that clonidine causes partial relaxation of rabbit aortic strips which are maximally constricted by epinephrine. Aars (1972) confirmed this effect in vivo. Clonidine has therefore been characterized as an alpha-adrenergic receptor agonist with partial antagonist activity (Ariens, 1964).

Sympathetic transmission can also be inhibited by interfering with the release of transmitter. Starke et al (1972) demonstrated that clonidine has the ability to decrease release of norepinephrine from post-ganglionic sympathetic nerves in response to stimulation. This effect was blocked by phenoxybenzamine (Starke and Altman, 1973). When the relative potency of various adrenergic agonists on pre- and post-synaptic receptors was compared, clonidine appeared to be a relatively potent pre-synaptic agonist (Starke et al, 1975). Data

from experiments in pithed rats (Armstrong and Boura, 1973) and dogs with sectioned spinal cords (Scriabine et al, 1970) also show that clonidine has some ability to depress peripheral sympathetic transmission. Although this action could contribute to the cardiodepressant action of clonidine, the concentration required to substantially reduce transmitter release is much higher than that achieved during administration of doses of clonidine which elicit decreases in blood pressure and heart rate (Werner et al, 1972).

Clonidine increases the firing rate of aortic baroreceptors (Aars et al, 1972). This could partially explain the decrease in blood pressure and heart rate resulting from clonidine treatment; however, the blood pressure and heart rate effects are not abolished by section of the aortic nerves. Korner et al (1974) observed that high doses of clonidine (20 ug/kg) caused the aortic baroreceptors to reset so that at a given arterial pressure the firing frequency was increased. Lower doses had no effect. Other investigators have demonstrated that high doses of clonidine inhibit firing of the sinus nerve in animals with carotid sinus pressure maintained at a constant level (Antonaccio et al, 1975). Carotid sinus denervation fails to alter clonidine's cardiodepressant effect (Magus and Long, 1968). Taken together, these data indicate that the primary mechanism of clonidine's depression of the cardiovascular system does not involve direct actions on the peripheral nervous system, or direct action on baroreceptor function.

The decreases in blood pressure and heart rate caused by clonidine are best explained by its central inhibitory effect on the sympathetic

nervous system and its facilitation of vagal activity. Schmitt et al (1967) demonstrated that concurrent with the hypotension and bradycardia caused by clonidine there was a striking decrease in sympathetic tone. The rate of discharge of the splanchnic and inferior cardiac nerves was decreased. Intravenous clonidine had this effect in dogs, cats and rats (Schmitt et al, 1968; Klupp et al, 1970; Haeusler, 1974a). Clonidine also decreases sympathetic tone in animals with denervated baroreceptors (Schmitt et al, 1967) and in DOC hypertensive rats (Bucher et al, 1973). In addition, clonidine prevents the increase in sympathetic nerve activity elicited by stimulation of the posterior hypothalamic pressor area in cats (Schmitt et al, 1968; Haeusler, 1973). Similarly, clonidine inhibits the increase in sympathetic tone caused by sciatic nerve and medullary stimulation (Schmitt et al, 1968; Sinha et al, 1973).

Kobinger and Walland (1972a) showed in pentobarbital anesthetized dogs that intravenous clonidine increases the reflex bradycardia in response to injections of hypertensive doses of angiotensin II or norepinephrine. In decerebrate rats (Kobinger and Pichler, 1974) and in conscious dogs (Walland et al, 1974) the same facilitation of the baroreceptor reflex response was seen.

Clonidine decreases sympathetic tone and increases the bradycardia produced when it is administered by routes which perferentially act on the brain. Intracisternal administration of low doses of clonidine (1 ug/kg) to vagotomized cats produced a greater reduction in blood pressure and heart rate than similar doses given intravenously

(Kobinger and Walland, 1972a). Intracisternal administration of low doses of clonidine also potentiate reflex decreases in heart rate more effectively than intravenous administration of the same dose (Kobinger and Walland, 1972a).

Magus and Long (1968) showed that clonidine acts primarily in the head to lower blood pressure and heart rate when they demonstrated that in decapitated cats, Clonidine did not decrease blood pressure and heart rate. A somewhat more elegant experiment, using the classic model of cross-circulation in dogs where the body of one animal supplies blood to the head of another, also showed that clonidine acts in the head to decrease blood pressure and heart rate (Sherman et al, 1969).

Dhawan et al (1975) demonstrated that infusion of low doses of clonidine into the lateral ventricle of cats inhibited the cardiovascular response to stimulation of hypothalamic, medullary and spinal vasomotor loci. Sattler and Van Zwieten (1967) demonstrated that low doses of clonidine (.25 - 2 ug/kg) administered into the vertebral artery of cats and dogs decreased blood pressure and heart rate. However, Laubie et al (1976a) reported that intravertebral clonidine administration had an unexpectedly small depressant effect on sympathetic nerve activity. Similar separation of the hypotensive and sympathodepressant effects of clonidine was seen in dogs with large lesions of the ventral surface of the medulla. Intravenous clonidine decreases blood pressure in these animals but has no effect on the firing rate of sympathetic nerves (Laubie et al, 1976a). These results are difficult

to explain if clonidine's hypotensive effect is ascribed to its depressant effect on sympathetic tone. Clonidine might have reduced activity in other sympathetic nerves than those which were recorded from in these experiments. Clonidine has been reported to differentially reduce the activity in various sympathetic nerves (Schmitt et al, 1975).

I.C. Mechanisms of Central Action

The demonstration that clonidine, an alpha-sympathomimetic agent in the periphery, has an important central action stimulated research on the mechanism by which this central action is mediated. Experiments in which clonidine and other pharmacological agents have been applied directly to neurons throughout the brain have yielded interesting results. Other investigators have tried to determine the nature of the central receptor on which clonidine acts by using pharmacological approaches.

I.C.1. Direct Application of Clonidine to Neurons in the Central Nervous System

Microiontophoretic application of clonidine to neurons in the medulla oblongata of cats decreased the firing rate of some of the neurons (Hukuhara et al, 1968). However, not all neurons identified as vasomotor neurons were influenced by clonidine, and the response of those neurons which were inhibited was not dose-dependent.

Anderson and Stone (1974) applied clonidine and norepinephrine to 247 neurons by microiontophoresis in the cerebral cortex and the medullary reticular formation of rats. 79% of the medullary neurons and 60% of the cortical neurons responded to both norepinephrine and clonidine in the same manner, usually with a depression of firing rate. This indicates that in many cases, clonidine and norepinephrine may be acting on the same receptors. The ionotophoretic application of clonidine decreased the firing rate in 13% of the cells in which the application of norepinephrine increased the firing rate. These differences could be explained if clonidine competes with endogenous norepinephrine for post-synaptic sites or acts to inhibit the release of endogenous transmitter. Svensson et al (1975) have demonstrated that direct application of clonidine to neurons in the locus coeruleus, one of the major catecholamine containing cell groups (see section I.D.), causes a depression in firing rates. Interestingly, intravenous administration of the catecholamine releaser, amphetamine, also caused depression of the firing rate of locus coeruleous neurons (Walters et al, 1974).

The neuronal responses to clonidine may be due to clonidine acting on receptors similar to those on which norepinephrine normally acts. It is therefore worth noting how microiontophoretic application of norepinephrine affects neurons. Johnson et al (1969) showed in rats that norepinephrine application usually excited cortical neurons. This excitation was blocked by phentolamine and propranolol. However, decreases in the firing rate of cortical neurons induced by norepinephrine

were not blocked by phentolamine, dibenamine, propranolol or sotalol. Cells which had both norepinephrine and isoproternol applied to them always responded to both agents similarly. This suggested that the receptor resembled a peripheral beta receptor (Johnson et al, 1969). Sharma (1977) also found that microiontophoretic application of norepinephrine to cortical neurons in rats caused increases in firing rate. However he concluded that norepinephrine is acting on an alpha-adrenergic receptor since phentolamine sometimes blocked its effect. Stone (1973) studied cells in the cortical pyramidal tract of the cerebral cortex of rats and found that direct application of norepinephrine usually depressed these cells. This action was occasionally blocked by phentolamine but most frequently was blocked by propranolol, supporting the earlier conclusion that in the cerebral cortex, norepinephrine acts on a receptor similar to a peripheral beta-receptor.

Boakes et al (1973) studied the responses of brain stem neurons to direct application of various adrenergic agents. They found that norepinephrine caused excitation of some neurons and depression of others. Neither the alpha-agonist phenylephrine or the beta-agonist isoproterenol mimicked the norepinephrine response. The alpha-antagonists phentolamine and phenoxybenzamine, and the beta-antagonist propranolol did not block these neuronal responses to norepinephrine. Based on the variability of neuronal responses to the direct application of different adrenergic agents it seems likely that the central receptor

on which norepinephrine acts is different from peripheral alpha and beta receptors.

I.C.2. The Pre-Synaptic Action of Clonidine in the Central Nervous System

Starke et al (1972) demonstrated that clonidine inhibited norepinephrine release from peripheral post-ganglionic sympathetic neurons. Previously Farnebo and Hamberger (1971) demonstrated that clonidine decreases the release of tritiated monoamines from field stimulated rat brain slices. Starke and Montel (1973) repeated and elaborated on these experiments. They showed that clonidine can decrease overflow of norepinephrine into the incubation medium and that it does so by inhibition of release rather than facilitation of metabolism or reuptake. They also demonstrated that the alpha-blockers phenoxybenzamine and phentolamine increased norepinephrine release, presumably by blocking the pre-synaptic inhibition of transmitter release by endogenous norepinephrine. Clonidine antagonized these effects of the alpha-blockers.

In vivo studies of the production of specific norepinephrine metabolites from tritiated dopamine in brain have yielded similar results (Braestrup and Nielsen, 1976). Clonidine decreases and alpha blockers increase norepinephrine release in vivo. Clonidine inhibits the effects of the blockers but the intraperitoneal injection of phenoxybenzamine, yohimbine or a high dose of aceperone did not block the clonidine effect. Earlier studies by Anden et al

(1970) also indicated that clonidine decreased total turnover of norepinephrine in the brain. Their experiments showed a partial blockade of this effect by phenoxybenzamine and by haloperidol.

I.C.3. The Relative Importance of the Pre-synaptic Action of Clonidine

The demonstration that clonidine can inhibit the release of norepinephrine in the central nervous system led to studies on the importance of this ability in mediating responses to clonidine. Various attempts were made to determine if clonidine still exerts its actions in animals in which central catecholamines were depleted. Haeusler (1974c) treated cats with reserpine and alpha-methyl-p-tyrosine to deplete norepinephrine. He showed that in these animals, intravenous clonidine decreased sympathetic nerve activity and inhibited the sympathetic discharge which occurs in response to hypothalamic stimulation. The reserpine and alpha-methyl-p-tyrosine pretreatment lowered blood pressure and heart rate. Clonidine had no further effect. These experiments indicated that clonidine could act in spite of norepinephrine depletion, arguing against a pre-synaptic site of action.

Dollery and Reid (1973) pretreated rabbits with intracisternal 6-hydroxydopamine and reported that it reduced the hypotensive action of clonidine. They therefore concluded that the presence of intact noradrenergic neurons is necessary for clonidine to exert its hypotensive effect. Finch et al (1975) treated rats with 6-hydroxydopamine, but in

contrast to Dollery and Reid, saw no change in clonidine's hypotensive action. Pretreatment with dopamine antagonists or the serotonin depleting drug, p-chloro-N-methyl amphetamine, had no effect on the hypotensive response to clonidine.

Kobinger and Pichler (1974) showed that in rats the facilitory effect of clonidine on the reflex bradycardia resulting from intravenous angiotensin II administration was not effected by pretreatment of animals with reserpine and alpha-methyl-p-tyrosine.

With the exception of Dollery and Reid's work (1973) all the studies indicate that clonidine can exert its effects in animals without intact central noradrenergic systems. This suggests that if clonidine is acting at the same loci as norepinephrine, its cardiodepressant effects are mediated via postsynatpic receptors.

I.C.4. Other Possible Mechanisms of Action of Clonidine in the Central Nervous System

In slices of rat cerebral cortex, low doses of clonidine and the related imidazoline oxymetazoline had no effect on basal cAMP content (Skolnick and Daly, 1975). However, they antagonized the norepinephrine stimulated increase in cAMP. They also antagonized the stimulation of cAMP formation produced by methoxamine (an alpha-agonist). Neither agent affected isoproterenol stimulated increases in cAMP (Skolnick and Daly, 1975). These results indicate that clonidine and oxymetazoline can function as antagonists of alpha-receptor mediated effects in brain tissue.

At much higher doses, clonidine stimulated accumulation of cAMP in guinea pig brain (Audigier et al, 1976). This effect was blocked by metiamide, an H_2 receptor antagonist. However, it seems unlikely that H_2 receptors are important in mediating clonidine's action since the levels which stimulate H_2 receptors are much higher than the levels which act in vivo to alter various physiological parameters (Audigier et al, 1976).

Several authors have shown that clonidine decreases turnover of serotonin (Maj et al, 1973; Rochette and Bralet, 1975). This effect appears to be indirect since direct application of clonidine to serotinergic neurons does not have any effect (Svenssan et al, 1975). It is possible that clonidine acts pre-synaptically to inhibit the release of serotonin. Clonidine has no effect on specific serotonin receptors in a snail model which has been a good model system for studying drug interactions with serotonin receptors (Woodruff et al, cited in Broekkemp and Van Rossum, 1972). Thus, clonidine does not appear to act on serotonin receptors but may indirectly influence the serotinergic system.

I.C.5. Effects of Receptor Blockade on the Decreases in Blood Pressure and Heart Rate Caused by Clonidine

In section I.C.1. the differences between the central and peripheral receptors which norepinephrine stimulate are discussed. These differences complicate any pharmacological attempt to determine if clonidine acts on similar noradrenergic receptors since the classic

receptor blockers are not always effective in blocking the central effects of norepinephrine (Johnson et al, 1969; Boakes et al, 1971; Philippu et al, 1971).

Various alpha-blockers have been used to determine if clonidine acts on alpha receptors in the central nervous system. Intravenous administration of phentolamine (Katic et al, 1972; Bucher et al, 1973) has been reported to block the hypotensive response to clonidine administration. Finch (1974) reported that intraventricular administration of phentolamine or tolazoline blocked the hypotensive response to clonidine administration. Finch (1974) reported that intraventricular administration of phentolamine or tolazoline blocked the hypotensive response to clonidine. However Schmitt and Schmitt (1970) demonstrated that these effects were only seen when the control blood pressure was already decreased by the blocking agent. They found that once blood pressure had returned to control levels, neither intravenous nor intraventricular phentolamine or tolazaline altered clonidine's effect on blood pressure. Intravenous or intraventricular administration of phentolamine or tolazoline was also ineffective in preventing the decrease in sympathetic nerve activity caused by clonidine (Schmitt and Schmitt, 1970). Walland et al (1974) and Kobinger and Walland (1972b) reported that phentolamine prevented clonidine's facilitation of the baroreceptor reflex. However, it is difficult to interpret these results since phentolamine decreased the control blood pressure and this could affect the baroreceptor response.

The lack of effectiveness of phentolamine and tolazoline is not particularly surprising since even in the periphery these agents do

not consistently block all the responses that involve alpha-adrenergic receptors (Nickerson and Collier, 1975).

Bolme and Fuxe (1971) demonstrated that intravenous phenoxybenzamine blocked the hypotensive and bradycardic effects of clonidine. Ganong et al (personal communication) also showed that intravenous phenoxybenzamine blocked the hypotensive effect of clonidine. Other investigators have not been able to confirm this result (Katic et al, 1972; Reid, I.A., personal communication). Ganong et al (1977) demonstrated that phenoxybenzamine injection into the fourth cerebral ventricle blocked the hypotensive response to clonidine. Phenoxybenzamine has also been reported to block clonidine's effect on norepinephrine turnover (Anden et al, 1970).

It is important to realize that phenoxybenzamine and the haloalkylamine family of alpha-blockers are not competitive blockers but interact with the receptor to inactive it. In vitro studies show that the log-dose response curve to an agonist is shifted progressively to the right by increasing doses of a haloalkylamine blocking agent, without a change in the slope or asymptote as is characteristic of competitive antagonist. This is possible with a noncompetitive blocker because most normal tissues contain "spare receptors" (receptors in excess of the number required for a maximal response to most agonists), and a large proportion of these can be inactivated before the tissue is incapable of a maximal response when exposed to adequate concentrations of an agonist. If the number of receptors is reduced below a certain level, then no concentration of agonist will produce a maximal response

(Nickerson, 1956). This phenomenom has been described in the case of phenoxybenzamine antagonism of the dose-dependent contraction of the rabbit aorta induced by clonidine. Phenoxybenzamine shifts the dose response curve to the right (Constantine and McShane, 1968).

It is worth noting that phenoxybenzamine also is a potent H_1 receptor blocker (Leonard and Huttrer, 1950) and has some ability to block serotonin receptors.

Thus phenoxybenzamine blockade suggests but does not prove that alpha-receptors are involved in mediating a response. In addition, unsuccessful blockade with phenoxybenzamine does not necessarily mean that alpha-receptors are not involved in mediating a response.

Of all the classic alpha-adrenergic receptor blocking drugs, piperoxan, a benzodioxan, has most consistently been reported to block clonidine's central effects. Piperoxan has variable effects on central blood pressure, heart rate, and sympathetic activity depending on dose and route of administration. Several investigators have reported that intravenous or intracerebroventricular administration of piperoxan can block or reverse clonidine's depressant effects on blood pressure (Schmitt et al, 1971; Bucher et al, 1973; Finch, 1974; Haeusler, 1974b) heart rate (Schmitt et al, 1971; Haeusler, 1974b) and sympathetic activity (Schmitt et al, 1971; Bucher et al, 1973; Haeusler, 1974b). However, the piperoxan block is overcome by administration of a slightly higher dose of clonidine (Schmitt et al, 1971; Bucher et al, 1973). Schmitt et al (1973) reported that piperoxan and yohimbine shifted the dose-response curve for the hypotensive response to intravertebral arterial clonidine to the right.

Piperoxan has been largely abandoned as a pharmacological tool because it has so many other actions in addition to alpha adrenergic blocking activity (Nickerson and Hollenberg, 1967). It would therefore seem unwise to draw conclusions about the receptor type mediating clonidine's effects based on piperoxan's blocking ability. Further complicating the issue are the recent results of Reid and Jones (1976), who report that piperoxan did not block clonidine's hypotensive effect in dogs.

Van Spanning and Van Zweiten (1973) and Van Zweiten (1976) demonstrated that in cats intravertebral arterial administration of tricyclic antidepressants reduced the hypotensive effects of clonidine injected by the same route. They ascribed this action to the alpha blocking activity of these agents. However, other investigators could not block the effect of intravenous administration of clonidine to rabbits with intravenous desipramine, a tricyclic antidepressant which Van Zweiten reported was effective (Hoefke and Warnke-Sachs, 1974).

Pimozide and spiroperidol, at doses which blocked central dopamine receptors but not norepinephrine receptors, did not block clonidine's hypotensive effect (Bolme and Fuxe, 1971). However, Bloch et al (1974) reported that when pimozide was applied directly to the ventral surface of the medulla oblongata it blocked the hypotensive effects of clonidine application to this area. The local concentrations of pimozide were not described and it is possible that pimozide was blocking other receptor types.

In conclusion, it seems likely that clonidine interacts with a receptor(s) in the central nervous system which is similar, but not identical, to peripheral alpha-receptors. As is the case with

norepinephrine, the central actions of clonidine cannot be ascribed to a single well described receptor type. There is considerable disagreement about the ability of various alpha-adrenergic blockers to alter responses to clonidine and norepinephrine. It is likely that at least one receptor action of clonidine involves receptors which are also stimulated by norepinephrine.

Evidence from experiments using pharmacological agents other than clonidine also suggests that a central receptor, different from peripheral alpha and beta receptors, is stimulated by the endogenous central catecholamines to cause decreases in blood pressure and heart rate similar to those produced by clonidine.

The catecholamine precursor L-dopa decreases blood pressure when given into the cerebral ventricles of dogs (McCubbin et al, 1960) and cats (Torchiana et al, 1973). If given after intravenous carbidopa, a decarboxylase inhibitor that does not cross the blood brain barrier (Clark et al, 1973), intravenous L-dopa also has a hypotensive effect (Kaplan et al, 1972; Minsker et al, 1971; Antonaccio et al, 1974). However, if L-dopa is given after RO 4-4602, a decarboxylase inhibitor which in large doses crosses the blood brain barrier and prevents central decarboxylation of L-dopa (Bartholini and Pletscher, 1969), there is no hypotensive response (Henning and Rubenson, 1970; Minsker et al, 1971). Thus, one of the decarboxylated metabolites of L-dopa has hypotensive actions in the central nervous system.

Another pharmacological agent, αmethyl norepinephrine which is known to stimulate alpha receptors, decreased blood pressure and

heart rate when administered in the third cerebral ventricle and these effects were antagonized by the alpha-blockers yohimbine and phentolamine (Heise and Kroneberg, 1972).

As mentioned previously central adrenergic receptors appear to be different from peripheral adrenergic receptors. Although cerebral blood vessels receive a rich sympathetic innervation (Purdy and Bevan, 1977) the receptors on them appear to differ from those on peripheral arteries. The basilar artery of the rabbit is about one thousand times less sensitive to norepinephrine than the saphenous artery. These arteries are equally sensitive to serotonin (Bevan et al, 1975). The cerebral arteries exhibit little stero-specificity in their constrictor response to d- or l-norepinephrine and the receptors appear to have much higher dissociation constants for phentolamine than the receptors in peripheral arteries (Duckles and Bevan, 1976). In addition, isoproterenol can produce "alpha-receptor" mediated vasoconstriction in the cat middle cerebral artery more effectively than the classic alpha-agonist phenylephrine (Edvinsson and Owman, 1974).

Electrical stimulation of isolated rabbit basilar arteries causes constriction, although the responses are small compared to peripheral responses (Bevan et al, 1975). Previous superior cervical ganglionectomy abolishes the response (Lee et al, 1976). The alpha-blockers phentolamine and phenxoybenzamine would be expected to block the response but actually augment the constrictor response to electrical stimulation. The same doses of alpha blocking drugs block the constrictor response to norepinephrine (Lee et al, 1976). Lee et al

(1976) conclude that these studies raise the question of whether neuromuscular transmission in arteries of the brain is mediated by the conventional adrenergic receptor mechanism. The same types of questions must be raised for other catecholamine-mediated responses in the central nervous system.

I.C.5. Central Sites of Action of Clonidine on the Cardiovascular System

Sattler and Van Zweiten (1967) demonstrated that clonidine acts in the area of the brain perfused by the vertebral artery to lower blood pressure. Since that time many investigators have tried to further localize the central sites of action of clonidine.

Katic et al (1972) administered clonidine into the vertebral and carotid artery of chloralose anesthetized dogs. They demonstrated that infusion of low doses of clonidine (.1 ug/kg/min) into the vertebral artery decreased blood pressure whereas intracarotid infusions had no effect. In these studies, a small increase in heart rate accompanied the fall in blood pressure resulting from intravertebral arterial infusion. Infusion of clonidine via the vertebral but not via the carotid arteries caused greater reflex decreases in heart rate in response to the injection of angiotensin II into the vertebral artery than before clonidine was injected (Katic et al, 1972). Clonidine injection via the vertebral arteries but not via the carotid arteries also prevented the reflex increases in heart rate and blood pressure in response to carotid occlusion (Katic et al, 1972).

Another approach to localizing the site of action of a compound in the central nervous system is to isolate parts of the nervous system and determine if the agent still has activity. In animals in which the brain stem was transected above the medulla oblongata, clonidine still lowered blood pressure, heart rate and the rate of sympathetic discharge (Schmitt and Schmitt, 1969; Sinha et al, 1973). Since clonidine had little if any effect in animals with transections below the medulla oblongata (Scriabine et al, 1970), this region presumably includes the primary site of action of clonidine on blood pressure, heart rate and sympathetic discharge.

Haeusler (1973, 1974a) showed that the cardiovascular response to clonidine was similar to the response to stimulation of the carotid sinus (baroreceptor or buffer) nerves. He suggested that clonidine's effects on the cardiovascular system are elicited by activating a central pathway that is part of the baroreceptor reflex. This hypothesis was supported by recordings of neuronal activity in the medulla oblongata showing that clonidine inhibits many neurons which receive baroreceptor projections (Korner et al, 1974).

In pentobarbital anesthetized dogs Laubie et al (1976a) found that clonidine potentiated the bradycardic response to sinus nerve stimulation but did not alter the bradycardic response to stimulation of the nucleus tractus solitarius or of the nucleus ambiguus. This suggested, as did Haeusler's results, that clonidine affected the baroreceptor reflex, probably at the first central synapse. However, Laubie (1976b) showed in cats that although lesions of the nucleus tractus solitarius abolished

clonidine's effect on the baroreceptor reflex, these lesions did not alter clonidine's blood pressure or heart rate lowering effects. Lipski et al (1976) also reported that clonidine did not potentiate cardiovascular reflexes in cats with bilateral lesions of the nucleus tractus solitarius. In dogs, bilateral lesions of the nucleus tractus solitarius and of the vagal motor nucleus induced a fulminating hypertension and bradycardia but the effects of clonidine on blood pressure and heart rate persisted (Laubie and Schmitt, 1977). In addition, De Jong (1974) demonstrated that bilateral norepinephrine injection into the nucleus tractus solitarius decreased blood pressure and heart rate. Clonidine administered in the same way was ineffective in depressing blood pressure or heart rate (DeJong; cited in Bousquet et al, 1975). These results all indicate that the nucleus tractus solitarius is not the primary site of clonidine action on resting blood pressure, heart rate or sympathetic discharge. However, clonidine may act in the nucleus tractus solitarius to alter baroreceptor reflex responses.

Although bilateral destruction of the motor vagus nuclei did not alter clonidine's cardiodepressant actions (Laubie and Schmitt, 1977), infusion of clonidine directly into these nuclei did cause decreases in heart rate and blood pressure (Sinha et al, 1975). Similar changes were induced by infusion of clonidine into the medullary reticular formation (Sinha et al, 1975).

Application of clonidine to a small area called the Schlafke zone or area 5 of the ventral surface of the medulla oblongata has

been reported to decrease blood pressure and heart rate in cats (Bousquet and Guertzenstein, 1973; Dhawan et al, 1975). Bousquet et al (1975) claimed that bilateral destruction of this area abolished the hypotensive response to intravenous clonidine. Other investigators could not confirm these results (Laubie et al, 1976b; Laubie and Schmitt, 1977). More extensive lesions in the area around the Schlafke zone of the ventral surface of the medulla, including a large portion of the associated reticular formation did abolish the decrease in heart rate and blood pressure normally produced by clonidine. However, resting sympathetic tone was decreased (Laubie and Schmitt, 1977). In dogs, destruction of this area decreased resting sympathetic discharges but did not alter the blood pressure and heart rate lowering effects of clonidine (Laubie and Schmitt, 1977). Although this evidence demonstrates that clonidine exerts its blood pressure and heart rate lowering effects or at least a portion of them by acting on structures in the medulla oblongata, the exact site(s) of action in the medulla oblongata is unclear. It is also possible that clonidine has effects on structures rostral to the medulla oblongata which either contribute to or buffer its blood pressure and heart rate lowering action.

Injection of clonidine into the posterior hypothalamus increases the pressor response evoked by hypothalamic stimulation (Phillippu et al, 1973). Shaw et al (1971a) demonstrated in conscious rabbits that pontine section caused clonidine to have a greater hypotensive effect. The bradycardic action was reduced in these animals. Another study indicating clonidine may act in rostral structures to increase blood

pressure showed that injection of clonidine into the lateral cerebral ventricles of cats caused hypertension if the aqueduct of Sylvius was cannulated to prevent the clonidine from reaching the fourth ventricle (Bousquet and Guertzenstein, 1973).

Trolin (1975) demonstrated that in conscious rats low doses of clonidine had no effect on blood pressure or heart rate. Decerebration and pentobarbital anesthesia increased resting blood pressure and heart rate and in these rats clonidine decreased blood pressure and heart rate. Higher doses of clonidine decreased blood pressure in the decerebrate animals. These results also suggest that the hypotensive response is located in the brain stem and that clonidine may be having hypertensive activity at suprabulbar levels.

The only evidence that clonidine acts in the rostral portion of the brain to decrease blood pressure and heart rate are studies that showed that high doses of clonidine injected into the anterior hypothalamus decreased blood pressure and heart rate (Struyker, Boudier and Van Rossum, 1972). However the potential problem of back-leakage of infused clonidine into the ventricle causes problems in interpreting these results.

I.D. Anatomy of the Central Noradrenergic and Adrenergic Systems

The evidence reviewed in the previous sections suggests that clonidine may not act on a classical, peripheral type alpha-receptor in the central nervous system. However, there are significant

similarities between the central physiological actions of clonidine and norepinephrine. Microiontophoretic techniques suggest that in many cases, clonidine and norepinephrine may be acting on the same type of receptor (Anderson and Stone, 1974). There is also evidence that clonidine may affect the noradrenergic cell bodies (Svensson et al, 1975). Since it appears that the central noradrenergic and adrenergic systems may be important in understanding the central actions of clonidine, it is necessary to consider the anatomical distribution of these systems.

Vogt (1954) first demonstrated that norepinephrine and epinephrine are present in mammalian brain and described their uneven distribution, concentrations being higher in medulla and hypothalamus than in other parts of the brain. The possibility that these monoamines may function as central neurotransmitters has stimulated further detailed research on their anatomical distribution and functions.

Falck et al (1962) developed a histochemical technique based on the fact that formaldehyde converts catecholamines to compounds which fluoresce. Using this technique, they provided the first evidence that the monoamines were localized intracellularly in the nervous system (Carlsson et al, 1962). Dahlstrom and Fuxe (1964) utilized the technique in combination with various pharmacological manipulations to localize monoamine neurons. They demonstrated that the monoamine containing neurons were located almost exclusively in the brain stem, and concluded that the numerous monoamine terminals in the brain derive from axons of these neurons. Fuxe (1965) later used this technique to describe the distribution of these neurons. Ungerstedt

(1971) combined the fluorescense method with lesion studies to provide a definitive description of the nuclei and distribution of monoamines in rat brain. His work has been confirmed and further details have been provided by the use of new techniques for dissection of specific nuclei (Palkovits, 1973) combined with sensitive assays for monoamines (Koslow et al, 1974; Koslow and Schlumpf, 1974) and for enzymes involved in monoamine synthesis (Brownstein et al, 1976). In addition, immunohistochemical localization of the monoamine synthetic enzymes has been very useful in describing these neuronal systems (Hokfelt et al, 1974).

Dahlstrom and Fuxe (1964) described groups of catecholamine containing cells in the brain and numbered them A1 through A13 in sequence as encountered from the caudal medulla moving rostrally to the hypothalamus. Ungerstedt (1971) uses this nomenclature in his description of the central noradrenergic system (Figure 1). The most caudal noradrenergic containing cell group, A1, gives rise to pathways which descend in the anterior and lateral funiculs and terminate in the ventral horn, dorsal horn and sympathetic lateral column of the spinal cord. The A1, A2, A5, and A7 cell groups in the medulla oblongata and pons project anteriorly to form one of two major ascending noradrenergic pathways, the ventral noradrenergic pathway or bundle. The axons ascend into the medial lemniscus and then continue rostrally, mainly in the medial forebrain bundle. These fibers then course rostrally into the midbrain and forward to innervate

Figure 1

Sagittal projection of the ascending NA pathways. The descending pathways are not included. The stripes indicate major sites of noradrenergic nerve terminals. (From: Ungerstedt, U., 1971)

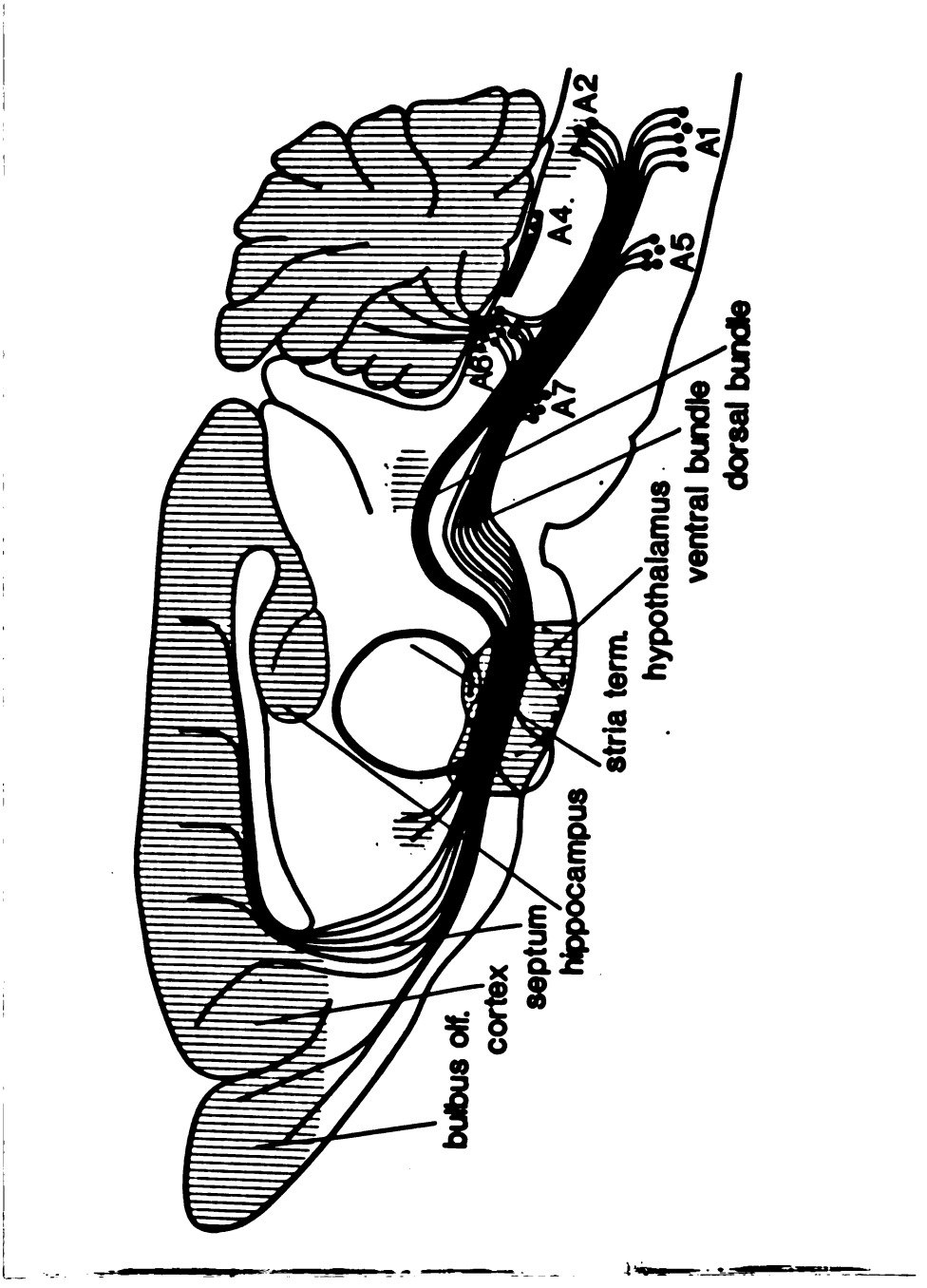
A2
A1
A4
A5
A3
A7
dorsal bundle
ventral bundle
hypothalamus
stria term.
hippocampus
septum
cortex
bulbus olf.

FIGURE 1

most of the hypothalamus, the bed nucleus of the stria terminales and other regions of the diencephalon. A1 cell groups also give rise to fibers which end in the dorsal motor nucleus of the vagus and the nucleus of the tractus solitarius.

The A6 group of cells is the locus coeruleus. It is located in the dorsomedial pons and gives rise to a descending pathway that overlaps the ventral noradrenergic pathway to lower brainstem nuclei. Fibers also project from it laterally to the cerebellum and rostrally to form the dorsal noradrenergic pathway or bundle. The axons in this bundle pass rostrally into the medial forebrain bundle and in the septum turn caudally into the cingulate gyrus. Along this path, branches are given off which innervate the cortex, hippocampus and amygdala.

Hokfelt et al (1974) used immunofluorescent techniques to localize phenyl-ethanolamine-N-methyl-transferase (PNMT), the enzyme which converts norepinephrine to epinephrine. They demonstrated the presence of cells in the central nervous system which contain epinephrine, presumably functioning as a transmitter (Figure 2). One cluster of epinephrine containing cells has a distribution and morphology identical to the A1 noradrenergic cell group of Dahlstrom and Fuxe (1964). Another group of epinephrine secreting cells duplicates in location and appearance the most rostral cells of the A2 noradrenergic group. Thus a few neurons, previously identified as catecholamine-containing cells using histochemical fluorescense methods, may synthesize epinephrine as their neurotransmitter.

Figure 2

Sagittal section of the rat brain showing the PNMT positive cell groups (C1 and C2) and the hypothetical adrenergic connections. (From: Hokfelt et al, 1974)

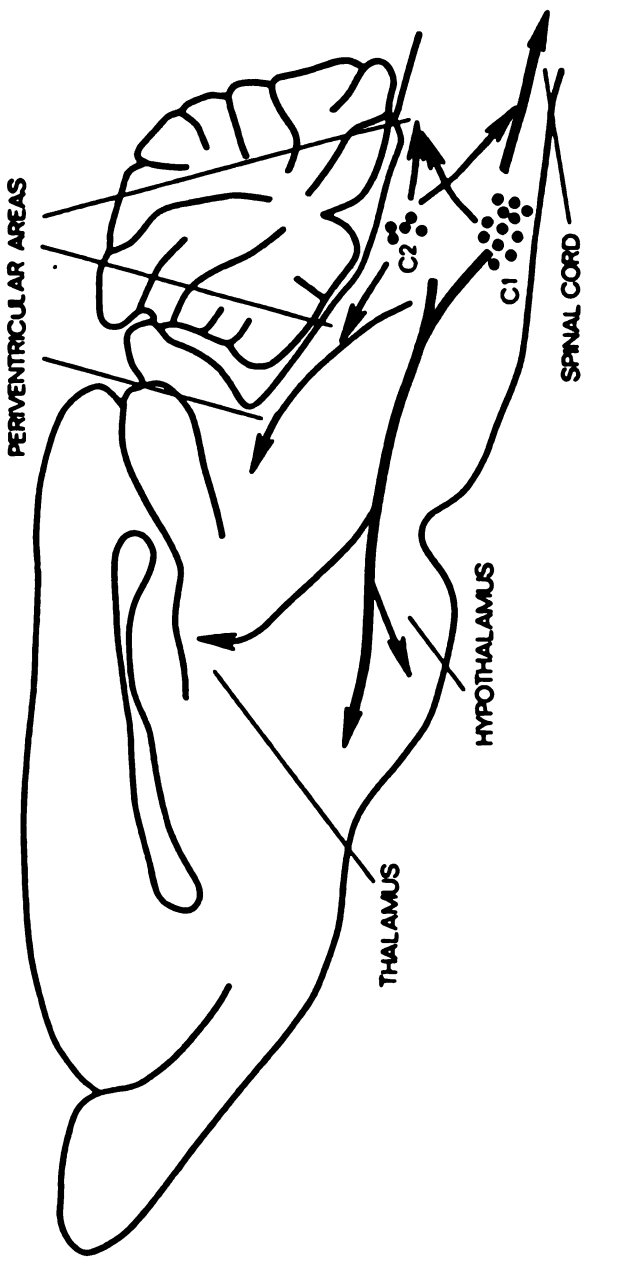
PERIVENTRICULAR AREAS
C2
C1
SPINAL CORD
HYPOTHALAMUS
THALAMUS

FIGURE 2

As described above, cells from A1 and A2 cell groups give rise to axons which descend in the spinal cord and some fibers which ascend in the ventral noradrenergic bundle. These fibers innervate areas in the medulla, pons, midbrain, hypothalamus and thalamus. There are also some epinephrine containing fibers from the A2 cell group (called "C2 cell group" when referring to PNMT containing cells) which innervate the A6 noradrenergic cells in the locus coeruleus.

Since the central neuronal systems described above contain neurotransmitters that also act on peripheral alpha-receptors, it is possible that clonidine, which acts on peripheral alpha-receptors, interacts with these systems either by inhibiting or stimulating neurons at the numerous sites to which the norepinephrine and epinephrine containing cells project.

I.E. The Effect of Clonidine on Renin Secretion

The early evidence that clonidine decreases blood pressure and heart rate and that it is an effective therapeutic agent in hypertensive patients has stimulated interest in the effects of clonidine on renin secretion. In both hypertensive patients and normal humans clonidine treatment decreases plasma renin activity (Hokfelt et al, 1970; Baer et al, 1971; Hedeland et al, 1972; Salvetti et al, 1973; Weber et al, 1976). It has been suggested that the renin lowering effects of clonidine enhance its effectiveness as an antihypertensive drug (Pals, 1975; Weber et al, 1976). These findings have stimulated interest in how clonidine decreases renin secretion.

Several factors have been shown to control renin secretion. Intrarenal vascular stretch receptors respond to decreases in afferent arterial stretch by increasing renin secretion (Skinner et al, 1963, 1964; Blaine et al, 1971; Blaine and Davis, 1971). It is improbable that clonidine acts to inhibit renin secretion via actions on these receptors since clonidine can decrease renin secretion in pentobarbital anesthetized dogs even when renal perfusion pressure decreases (Onesti et al, 1971). These pressure decreases should cause renin increases.

Another factor controlling renin secretion is the rate of delivery of sodium chloride to the distal tubule. Receptors associated with the macula densa respond to decreases in sodium delivery by causing increases in renin secretion (Vander and Luciano, 1967). Clonidine causes changes in renal hemodynamics and renal function, but these changes should decrease sodium delivery to the macula densa region and thus increase renin secretion. Intravenous administration of clonidine to dogs decreases renal blood flow (Laubie and Schmitt, 1969; Barac, 1971; Chrystans and Lavender, 1973). Bolme et al (1975) found that in monkeys intravenous and intracerebroventricular clonidine administration caused slight increases in renal blood flow or had no effect. Similar responses have been demonstrated in humans (Reubi et al, 1970; Onesti et al, 1971).

Renal plasma flow is reduced by intravenous clonidine (Onesti et al, 1971; Chrystans and Lavender, 1975). Glomerular filtration rate is reported to decrease slightly (Hoefke and Kobinger, 1966;

Onesti et al, 1971) or not to change (Chrystans and Lavender, 1975). Sodium excretion has been found to decrease (Onesti et al, 1971; Chrystans and Lavender, 1975), increase (Olsen, 1976) or not to change (Reid et al, 1975) in response to clonidine. Potassium excretion has been reported to increase (Reid et al, 1975) or not to change (Chrystans and Lavender, 1975). None of these effects could explain the action of clonidine on renin secretion.

Angiotensin II and vasopressin have been shown to inhibit renin release (Bunag et al, 1967; Vandongen, 1975). Since clonidine decreases renin secretion, angiotensin II levels in plasma fall. The fall would tend to increase rather than decrease renin secretion. The plasma concentrations of vasopressin also decreases after clonidine administration (Humphreys and Reid, 1975) so changes in vasopressin levels cannot explain clonidine's effects on renin secretion. In two hypophysectomized dogs clonidine decreased plasma renin activity, indicating that clonidine's effects on pituitary hormones are not important in mediating its renin depressant action (Reid et al, 1975).

Renin secretion is also affected by sympathetic nerves which have endings on the renal arteries and which probably synapse directly on the renin-secreting juxtaglomerular cells (Wagermark, 1968). Catecholamine infusion into the renal artery (Wathen et al, 1965; Winer et al, 1971) or stimulation of the renal nerves (Loeffler et al, 1972; LaGrange, 1973) increases renin secretion. Renal nerve

stimulation or infusion of catecholamines into the renal artery could cause increases in renin secretion by a direct action on the juxtaglomerular cells or by altering renal hemodynamics. Johnson et al (1971) demonstrated that in dogs with nonfiltering kidneys, catecholamines increased renin secretion after papaverine was infused into the renal artery. In this preparation the macula densa and baroreceptor mechanisms are thought to be eliminated so the results suggest that catecholamines can stimulate renin release directly.

Renin secretion can be increased by stimulating "pressor areas" in the medulla oblongata (Passo et al, 1971), pons (Richardson et al, 1974), or midbrain (Ueda et al, 1967). It also can be decreased by stimulation of hypothalamic areas which decrease sympathetic tone and blood pressure. In animals with denervated kidneys renin secretion decreased (Zehr and Feigl, 1973). In these animals, hypothalamic stimulation did not cause further decreases in renin secretion (Zehr and Feigl, 1973).

These results demonstrate that sites in the central nervous sytem can affect renin secretion, presumably by altering renal nerve activity. Clonidine administration decreases the activity in the renal nerves (Bucher et al, 1973; Haeusler, 1974a; 1974b). It therefore seemed likely that the renin depressant effects of clonidine resulted from its causing a decrease in a tonic sympathetic stimulation of renin secretion.

Experiments which are similar in design to those used to determine the site of the blood pressure and heart rate lowering actions of

clonidine have been performed to localize the site at which it acts to supress renin secretion. Onesti et al (1971) showed that intracisternal administration of low doses of clonidine to pentobarbital anesthetized dogs decreased renin secretion. Reid et al (1975) administered clonidine into the third cerebral ventricle of pentobarbital anesthetized dogs at doses which had no effect when given intravenously. By controlling renal perfusion pressure the renin stimulatory effects of a decrease in blood pressure resulting from clonidine administration were prevented. They demonstrated that clonidine could act centrally to decrease renin secretion. Renal denervation decreased plasma renin activity and clonidine did not lower it further. Ganglion blockade also decreased plasma renin activity in pentobarbital anesthetized dogs. Clonidine had no further lowering effect on plasma renin activity (Nolan and Reid, 1978).

In dogs with spinal cord transection, intravenous clonidine also does not affect plasma renin activity (Ganong et al, 1978). However it still can decrease plasma renin activity in decerebrate dogs (Ganong, unpublished results) suggesting that clonidine exerts its action on renin secretion in the brainstem.

It has been suggested that clonidine may also have peripheral actions which contribute to its depressant effect on renin secretion. Pettinger et al (1976) have reported that in rats, high doses of clonidine decrease serum renin activity. This also occurs following blockade of autonomic ganglia. Unfortunately, these decreases could result from the hypertensive effects of peripheral clonidine administration.

An increase in blood pressure would be expected to decrease renin secretion.

Vandongen and Greenwood (1975) demonstrated that clonidine inhibited renin secretion from isolated perfused rat kidneys. However in a more stable isolated kidney preparation in which perfusion pressure is controlled, Fray et al (in preparation) were unable to confirm these results. In another in vitro preparation, incubated kidney slices, Nolly et al (1974) could not demonstrate any effect of clonidine on renin secretion. Thus it appears that the primary site of action of clonidine on renin secretion is in the central nervous system. Pharmacological blocking agents such as phentolamine and piperoxane are unable to block clonidine's effects on renin secretion (Nolan and Reid, submitted for publication).

These results are similar to those for blockade of the cardiodepressant effects of clonidine. As discussed in section I.C.5. clonidine may act on a central adrenergic receptor different from the peripheral receptors. Increasing central catecholamine concentrations by administering L-Dopa in combination with the peripheral Dopa decarboxylase inhibitor, Caribidopa, decreases blood pressure, heart rate and plasma renin activity (Blair et al, 1977). Thus, clonidine has the same effect on these variables as increasing central catecholamines. This again suggests that clonidine may act on a central receptor similar to the receptor which the central endogenous catecholamines effect.

I.F. The Effects of Clonidine on the Secretion of Pituitary Hormones

In previous sections results have been discussed which demonstrate that clonidine acts on a central "adrenergic-like" receptor to decrease blood pressure and heart rate. These findings led to clonidine's use as an agent for the investigation of the neurotransmitters involved in the control of some pituitary hormones. The results of these experiments cannot be properly interpreted without considering other work on neurotransmitter involvement in the control of each hormone. Therefore, in the review of clonidine's action on several pituitary hormones, a brief discussion of other work on neurotramsitter involvement in the control of each hormone is included.

I-F-1. The Effect of Clonidine on ACTH Secretion

Using adrenal venous corticoid secretion as an index of ACTH secretion, Ganong et al (1976) demonstrated that clonidine inhibits the increase in ACTH secretion produced by laparotomy stress in pentobarbital anesthetized dogs. They also showed that when clonidine (5 ug/kg) was administered into the third cerebral ventricle, there were decreases in the ACTH response to stress. This decrease was greater than the decrease in response to stress which resulted from intravenous administration of a larger dose (30 ug/kg) two hours later to the same dogs. These results indicate that clonidine acts in the central nervous system to depress the ACTH response to surgical stress. Ganong et al (1976) also demonstrated that clonidine decreased resting

plasma corticoid levels in pentobarbital anesthetized dogs. Holland et al (1978) administered clonidine to conscious dogs and could not measure any decreases in corticoid levels, but all their values were below the lower limits of their assay method.

Ganong et al (1976) also concluded that administration of phenoxybenzamine into the third cerebral ventricle, but not intravenously blocked clonidine's depressant effect on plasma corticoid concentrations. Phentolamine however, did not block clonidine's effect.

Chambers and Brown (1976) reported that in unanesthetized monkeys clonidine did not lower resting corticoid levels (Chambers and Brown, 1976) although the levels were already close to the lower limits of the assay method. Thus clonidine may not effect resting corticoid levels in conscious animals but does decrease corticoid levels in pentobarbital anesthetized animals.

Similar conflicting results between anesthetized and conscious animals have also been described with L-dopa treatment. In pentobarbital anesthetized dogs, L-dopa decreased the corticosteroid response to laparotomy stress. L-dopa in combination with the peripheral decarboxylase inhibitor, carbidopa, had the same inhibitory effect, whereas L-dopa in combination with RO-44602, a peripheral and central decarboxylase inhibitor, had no effect (Ganong, 1977b). This suggests that a decarboxylated derivative of L-dopa acts in the central nervous system to inhibit ACTH release. In conscious dogs, Holland et al (1978) showed that L-dopa increased corticoid levels. Carbidopa did not block this effect, but a dopamine receptor blocker, pimozide, converted the response to L-dopa to inhibition. These results can be explained by

the hypothesis that in the conscious dog L-dopa stimulates ACTH secretion by increasing dopamine secreiton. This effect may mask the inhibitory effect on ACTH secretion of increasing central norepinephrine content and release.

Administration of catecholamine releasing agents to dogs (Van Loon et al, 1971a) and to monkeys (Marantz et al, 1976) depressed corticoid levels. The dopamine receptor blocker, pimozide, did not block this effect in monkeys (Marantz et al, 1976) indicating that another catecholamine, possibly norepinephrine, mediated the response.

Intracerebroventricular administration of large doses of norepinephrine to dogs inhibited the ACTH response to the stress of laparotomy. The L-isomer was more effective than the D-isomer which suggests that the effect is specific (Van Loon et al, 1971a).

There is evidence in rats that direct application of norepinephrine to the median eminence and other selective sites in the central nervous system can increase ACTH secretion (Kreiger and Kreiger, 1970). However, Jones et al (1976) demonstrated that norepinephrine inhibited CRF release from the rat hypothalamus in vitro. Rose et al (1977) demonstrated that stimulation of sites in the brain stem of the dog from which noradrenergic fibers project to the hypothalamus, caused inhibition of the ACTH response to the stress of laparotomy. It appears that the predominant action of central norepinephrine in the rat and other species is to inhibit ACTH secretion and the ACTH response to various stresses (Scapagnini and Preziosi, 1973; Van Loon, 1973). It is possible that the inhibitory effect of clonidine on ACTH

secretion is mediated by an action on the same receptor type upon which endogenous norepinephrine acts.

I-F-2. Effects of Clonidine on Growth Hormone Secretion

Clonidine has been reported to stimulate growth hormone (GH) release in humans (Lal et al, 1975). Intravenous administration of clonidine increased plasma GH levels in pentobarbital anesthetized dogs (Lovinger et al, 1976) and in conscious dogs (Holland et al, 1978). Clonidine also increased GH secretion in monkeys (Brown et al, 1973).

A much lower intravenous dose is required to stimulate GH in the conscious dog than in the anesthetized dog. Surprisingly, in the anesthetized dog, administration of clonidine into the third cerebral ventricle at doses which elicit all of clonidine's other central effects does not increase GH secretion (Lovinger et al, 1976). However, administration of larger doses of clonidine (5 ug/kg) into the fourth cerebral ventricle of anesthetized dogs stimulated GH secretion (Ganong et al, unpublished observation). Nevertheless, if phenoxybenzamine was given into the third cerebral ventricle, it blocked the effects of intravenous clonidine on GH secretion (Ganong, 1977a).

Other evidence suggests that norepinephrine stimulates GH secretion. L-dopa administration increased plasma GH levels in both conscious (Takahashi et al, 1973; Holland et al, 1978) and pentobarbital anesthetized dogs (Lovinger et al, 1976). Carbidopa abolished the GH response to L-dopa in conscious dogs (Holland et al, 1978) and attenuated the

response in anesthetized dogs (Lovinger et al, 1976). These results suggest that at least in the conscious dog, increases in catecholamines outside the blood brain barrier cause increases in GH, in response to L-dopa administration. Takahashi et al (1973) also reported that intravenous dopamine, norepinephrine and epinephrine stimulate GH secretion. Since these substances do not cross the blood brain barrier, it appears that catecholamines can increase GH at a site outside the blood brain barrier. The GH response to L-dopa and the other catecholamines is attenuated by intravenous administration of the alpha antagonist, phentolamine in conscious dogs (Takahashi et al, 1973). In pentobarbital anesthetized dogs, administration of phenoxybenzamine or phentolamine into the third cerebral ventricle blocks the GH response to intravenous L-dopa (Lovinger et al, 1976). These results suggest that in conscious and anesthetized dogs alpha-adrenergic stimulation increases GH secretion. Ruch et al (1977) demonstrated that in conscious cats intravenous L-dopa increased GH secretion. Peripheral decarboxylase inhibition abolished the hyperglycemic effects of L-dopa but did not alter L-dopa's effect on GH. Phenoxybenzamine abolished the GH stimulatory effect of L-dopa. In addition, direct injection of norepinephrine into the anterior and medial hypothalamus caused increases in GH secretion. These results suggest that in the cat central norepinephrine stimulates GH secretion. In baboons, microinjection of norepinephrine into the ventromedial nucleus of the hypothalamus increased GH secretion (Toivola and Gale, 1972).

In conclusion, catecholamines may affect GH secretion at two or more sites. One site is outside the blood brain barrier and is not very responsive in pentobarbital anesthetized animals. The site inside the blood brain barrier is important in anesthetized animals. It may play a role in conscious cats. The site or sites at which clonidine acts to stimulate GH is unclear. Clonidine's effect on GH is blocked by administration of alpha blockers in the third cerebral ventricle in anesthetized dogs but it is difficult to elicit a stimulatory GH response by intraventricular administration of clonidine in anesthetized dogs.

I-F-3. Effects of Clonidine on Vasopressin Secretion

Clonidine has been reported to have a diuretic effect in dogs (Chrystans and Lavender, 1973; Humphreys and Reid, 1975) and in hypertensive humans (Pettinger et al, 1977). Renal arterial administration of clonidine did not cause diuresis (Chrystans and Lavender, 1975). Humphreys and Reid (1975) showed that hypophysectomy prevented the diuretic response to clonidine. From these results they concluded that the diuretic effects of clonidine were due to an inhibitory effect on vasopressin secretion. They suggested that clonidine inhibited vasopressin by altering the peripheral circulation in a manner similar to norepinephrine; i.e., by increasing carotid sinus pressure (Berl et al, 1974).

Later studies in which vasopressin was measured directly by radioimmunoassay (Reid et al, submitted for publication) have demonstrated

that intravenous clonidine administration decreases the plasma levels of vasopressin. It has also been shown that the pressor activity of clonidine is not responsible for the inhibition of vasopressin secretion. Following peripheral alpha-blockade there was virtually no pressor response to clonidine but vasopressin still was decreased (Reid, personal communication). Combined treatment with L-dopa and the peripheral decarboxylase inhibitor, carbidopa, also decreased vasopressin levels (Blair, Reid and Keil, unpublished observation). Taken together, these data suggest that clonidine acts centrally to inhibit vasopressin release. However, direct evidence for a central action on vasopressin secretion is not available.

I.G. Other Actions of Clonidine in the Central Nervous System

Clonidine has marked sedative effects (Hoefke and Kobinger, 1966). Extensive studies of this effect have been performed on chickens (Fugner and Hoefke, 1971; Delbarre and Schmitt, 1969, 1971, 1973; Holman et al, 1971). Some, but not all, alpha-blockers prevent clonidine from exerting its sedative action. Marley and Nistico (1974, 1975) showed that intraventricular injection or intrahypothalamic injection of clonidine or norepinephrine in chickens caused sedation and sleep. These effects were reported to be blocked by both phentolamine and phenoxybenzamine.

Relatively high doses of clonidine have been reported to decrease the intake of food (LeDovarec et al, 1972) and water (LeDovarec, 1971a; 1971b) in rats. Phentolamine and piperoxan, but not phenoxybenzamine, blocked these effects.

High doses of clonidine also caused hypothermia in rats (Loverty and Taylor, 1969). In rats, intracisternal and intrahypothalamic administration of low doses of clonidine induced hypothermia (Tsoucaris-Kupfer and Schmitt, 1972). This effect was also blocked by selected alpha-blockers.

I.H. The Central Site of Action of Clonidine on ACTH, GH and Renin Secretion

In the preceding discussion the experimental literature relating to some of the actions of clonidine has been reviewed. The effects of clonidine on the cardiovascular system have been extensively studied. It is evident that the blood pressure effects of clonidine are primarily elicited by an action in the hindbrain although the exact site of action remains unclear.

Although the effect of clonidine on ACTH, GH and renin secretion appear to be mediated by an action on the central nervous system, few attempts to define the sites at which clonidine alters the secretion of these hormones has been reported. The remainder of this dissertation reports the results of experiments performed to determine the site of action of clonidine on ACTH, GH and renin secretion.

As is discussed in the following section (section I.H.) the forebrain and hindbrain are perfused by different arteries. By infusing clonidine into these different vascular supplies one can perfuse separate parts of the brain. Sattler and Van Zwieten (1967) used this approach to produce data which led them to the conclusion that clonidine's hypotensive action is elicited in the

hindbrain. A similar approach has been used to examine where clonidine acts to alter the secretion of ACTH, GH and renin. The distribution of the vessels supplying the brain in the dog has also been carefully studied.

I.I - The Distribution of the Vertebral Arteries and of the Carotid Arteries in the Brain

The major arteries which supply the brain are interconnected by communicating vessels (Willis, 1684). It is therefore impossible to determine the actual distribution of the individual arteries from anatomical descriptions. Kramer (1912) first attempted to determine the regions of the brain perfused by the vertebral arteries and the carotid arteries by injecting methylene blue dye through small needles into the arteries of dogs. He showed that dye injected into the vertebral arteries was distributed to the spinal cord, the medulla oblongata, the pons, the midbrain, the mamillary body and to the posterior portions of the cortex. Dye injected into the carotid arteries stained the entire cerebral cortex on the injected side except for the posterior part which was stained with vertebral arterial dye injection. Carotid dye injection also stained the optic chiasm, the infundibulum, both caudate nuclei and other anterior structures. In monkeys, Kramer (1912) found that the vessels supplied similar regions to those in the dog, except that in monkeys the carotid did not supply as much of the posterior cortex as it did in the dog. Jewell and Verney (1957) used similar methods and found that sometimes in dogs some carotid blood appeared

to perfuse the hindbrain. They explained that this was due to carotid arterial blood passing through an occipital artery-vertebral artery anastomosis, which is well developed in the dog (Jewell, 1952). This conclusion was based on the finding that in one dog in which the occipital artery was tied, no dye from a carotid injection reached the hindbrain.

Similar dye injection methods have been used to study the carotid arterial and vertebral arterial distribution in other species. In rabbits, dye injection combined with direct visualization of the basilar artery, suggests that blood from the carotid arteries and blood from the vertebral arteries passes into but not through the posterior communicating arteries (McDonald and Potter, 1951). In both sheep (Daniel et al, 1953) and goats (Andersson and Jewell, 1956) blood from the carotid artery perfuses the entire brain except for the medulla oblongata which receives blood from the vertebral arteries. In cats the carotid arteries perfuse the entire brain except for the pons and medulla (Holmes et al, 1957). Dye injection into the carotid arteries of cats during direct visualization of the basilar artery shows that the dye forms a fluctuating boundary in the rostral basilar artery. Injection of dye into the vertebral artery results in a distribution boundary in a similar location (Holmes et al, 1957). In rats, dye injected into the vertebral arteries mixes with dye injected into the carotid artery in the caudal end of the basilar artery (Wellens et al, 1976).

Microsphere studies of carotid artery and vertebral artery distributions in dogs and cats support most of the conclusions from

dye injection studies (Reneman et al, 1974; Wellens et al, 1975). In the cat most structures rostral to the midbrain receive a higher proportion of spheres injected into the carotid than into the vertebral arteries. The major difference between dogs and cats is that in cats the carotid arteries perfuse the posterior thalamic and posterior hypothalamic areas, whereas in dogs very little carotid blood is supplied to this region (Wellens et al, 1975). Injection of microspheres into the vertebral and carotid arteries of rats confirm the findings that the carotid arteries perfuse most of the rat brain (Wellens et al, 1976).

To take advantage of the differential distributions of the carotid and vertebral artery to make conclusions about the sites of action of clonidine, it was important to determine what parts of the brain were perfused by the carotid arteries and the vertebral arteries in the experimental models we studied. Although Wellens et al (1975) had used microspheres to study the carotid artery and vertebral artery distributions in the dog, their methods differed from the methods we used in several possibly important respects. In our studies the carotid and vertebral arteries were directly cannulated. Wellens et al (1975) introduced cannulas into the carotid artery through a branch. They examined vertebral arterial flow by cannulating the subclavian artery and tying off all branches distal to the vertebral artery. This required that the thorax be opened. In addition, Wellens et al (1975) only looked at distributions after the unilateral injection of microspheres whereas we examined the distribution of both vertebral and both carotid arteries.

It is also possible that the catheters used in the experiments described here could alter blood distribution patterns by partially occluding vessels. We examined the distribution of the vertebral and carotid arteries in our experimental preparations. We also determined the distribution of microspheres injected into the vertebral or carotid arteries after clipping of the basilar artery.

Section II

MATERIALS AND METHODS

II-A. Preparation

Male and female mongrel dogs ranging from 10 to 25 kg. in body weight were used in body weight were used in all of these studies. The dogs were anesthetized with sodium pentobarbital (30 mg/kg). Anesthesia was maintained throughout the surgical preparations and subsequent observations by administering additional anesthetic when needed. Rectal temperature was monitored and was maintained at 39° C by using a heating pad when necessary. An endotracheal tube was inserted and the dogs were maintained on a respirator.

Cannulas were inserted in one femoral artery and vein in every dog. In almost all experiments plasma renin levels were monitored. In order to control renal perfusion pressure a Blalock clamp was placed around the descending aorta proximal to the origin of the renal arteries. A cannula was also inserted into the brachial artery in order to monitor cardiovascular changes above the clamp. The femoral artery cannula allowed monitoring of pressures below the aortic clamp. Renal perfusion pressure was maintained at a constant level of about 95 torr throughout the experimental observations by appropriate adjustments of the aortic clamp. Blood pressure and heart rate were monitored using a Statham P-23 pressure transducer and a Grass Model 7 Polygraph.

Depending on the experimental protocol, both vertebral arteries, both carotid arteries, or all four arteries were cannulated. The

vertebral arteries were exposed for cannulation through an incision along the inferior margin of the sternocleidomastoid muscle. Blunt dissection around the sternocleidomastoid muscle and between the scalenus anterior muscle and longus colli muscle exposed the vertebral artery between its origin from the first part of the subclavian artery and its entrance on each side into the foramen in the transverse processes of the upper cervical vertebrae. The carotid arteries were easily located laterally to the trachea through the same incision.

The vertebral and carotid arteries were cannulated using a method which does not seriously disturb blood flow through the vessel (Rudolph et al, 1956). A small catheter (0.15 mm diameter) was passed approximately 1-3 cm. into the artery through a small hole in the arterial wall. It was secured in place by a purse-string suture which was earlier placed around the desired location of the arterial incision. The suture passed into but not through the arterial wall. The cannulation usually required occlusion of the artery for approximately one minute; however, sometimes up to five minutes of occlusion were required.

In some dogs a neurosurgical vascular clip was placed on the midpontine portion of the basilar artery. The basilar artery was approached through the mouth (Gildenberg and Ferrario, 1977). A midline incision was made through the soft palate and the naso-pharyngeal mucosa. Using a high speed dental drill, a hole was cut in the clivus above the pons. The dura was cut and a clip was placed on the basilar artery. The hole was then covered with gelfoam and bone wax.

In another group of dogs a cannula was passed from one femoral artery into the left atrium. Left atrial administration of microspheres allows determination of regional blood flows if reference samples are taken (Heymann et al, 1977). In these dogs the effects of clipping the basilar artery on cerebral regional blood flows was evaluated.

II-B. Hormone Assays

Ten ml. blood samples were collected from the femoral artery in chilled heparinized plastic centrifuge tubes. The samples were promptly centrifuged at 4° C and the plasma separated and frozen for subsequent assay. 11-hydroxycorticosteroid (corticoid) concentration was measured by the competitive protein binding method (Murphy, 1967). Plasma ACTH (Dallman et al, 1974) and growth hormone (Lovinger et al, 1974) were measured by radioimmunoassay. Blood for measurement of plasma renin activity (PRA) was collected in separate chilled centrifuge tubes containing ethylenediaminetetracetic acid and PRA was measured by radioimmunoassay of generated angiotensin I (Stockigt et al, 1971).

II-C. Experimental Protocols

At least ninety minutes after the completion of the surgical preparations, two control blood samples were taken twenty minutes apart. Clonidine was then injected into various vessels depending on the particular experiment. The clonidine was dissolved in 2.0 ml. of isotonic saline and the total dose was infused over a two minute

period at the rate of 0.1 ml/min using a Harvard infusion pump. When clonidine was injected into the vertebral or carotid arteries the total dose was split; half being injected into each side. In such cases 0.5 ml/min of the clonidine solution was infused into each of the vertebral arteries or into each of the carotid arteries over the 2 minute infusion period. The infusion of such a small volume of saline solution into a vessel with a minimal flow of one hundred times the infused volume should not affect the intra-arterial pressure and therefore should not alter the distribution pattern of the artery. Arterial blood samples were taken 15, 30 and 60 minutes following the beginning of the infusion. In some experiments an additional sample was taken 45 minutes after the infusion of clonidine. In the first series of experiments clonidine was administered in different vessels at 0 and at 60 minutes. Additional arterial blood samples were then taken at 75, 90 and 120 minutes after the first treatment. At the completion of many experiments radioactively labelled microspheres were injected (see section II.E.) to provide information about the cerebral arterial distributions. Heart rate, systolic blood pressure and diastolic blood pressure were recorded at each sample time.

Five dogs received a low dose of clonidine (0.5 ug/kg) via the carotid arteries. Sixty minutes after the carotid infusion of clonidine these animals received the same dose of clonidine into the vertebral arteries. Microspheres were injected into three of these animals via both carotid and both vertebral arteries. Two other dogs were surgically prepared in a similar manner and had microspheres injected but did not receive clonidine.

Clonidine (2 ug/kg) was infused into the carotid arteries of nine animals in which the carotid arteries but not the vertebral arteries were cannulated. Microspheres were injected into seven of these animals

via the carotid arteries at the end of the experiment.

Clonidine (2 ug/kg) was infused into the vertebral arteries of eight dogs in which the vertebral arteries but not the carotid arteries were cannulated. Microspheres were injected into two of these animals into the vertebral arteries at the end of the experiment.

In six dogs the basilar artery was clipped and the carotid arteries were cannulated. Clonidine (2 ug/kg) was infused into the carotid arteries. Microspheres were injected into the carotid arteries of all these animals at the completion of the experiment.

In seven dogs the basilar artery was clipped and the vertebral arteries were cannulated. Clonidine (2 ug/kg) was infused into the vertebral arteries. Microspheres were injected into both vertebral arteries of all these animals at the completion of the experiment.

Seven dogs received intravenous injections of clonidine (2 ug/kg). A volume of 2 ml of clonidine dissolved in isotonic saline was infused into the femoral vein. A Blalock clamp was placed around the aorta of these animals to control renal perfusion pressure but no neck surgery was performed.

To evaluate the effect of clipping the basilar artery on regional blood flows two sets of microspheres were injected into the left atrium before clipping the basilar artery in four dogs. Another two sets of spheres were injected after clipping the basilar artery. Reference blood samples for calculation of blood flows were taken from the brachial artery. Clonidine (30 ug/kg) was injected intravenously in two of these animals at the completion of the flow studies.

II.D. Distribution Studies

In some animals the distribution to the brain of the arteries which were cannulated was studied by injecting radionuclide-labelled plastic microspheres (Heymann et al, 1977) through the inserted cannula. These particles lodge in the microcirculation of the areas supplied by the arteries and their distribution can provide quantitative information on the arterial distribution. Certain criteria must be satisfied in order for this data to be meaningful:

(1) The spheres must be adequately mixed in the lumen of the artery so their distribution is a reflection of the distribution of the entire artery, not just a part of it. Mixing is particularly important if the method is being used to determine blood flows since reference samples from the arterial system are used to determine the number of spheres an organ receiving a known quantity of blood would contain. In the studies described here, the local injection of microspheres could result in inadequate mixing of the microspheres within the artery. Although this is possible, the distance from the site of injection to the brain is probably sufficiently large so that mixing occurred. This is even more likely since some turbulent flow (which encourages mixing) is produced by the cannula as well as by the actual injection of the spheres.

(2) It is also important that the microspheres are sufficiently large so that they lodge in the microcirculation of an area. Spheres fifteen micron in diameter were used in these studies. It has been demonstrated that these are appropariate for studying total and

regional cerebral blood flows (Marcus et al, 1976) so they are also adequate for the present distribution studies.

(3) The final criterion which must be satisfied is that sufficient numbers of spheres must be injected so that in each area perfused by an artery, the number of spheres perfused reached levels which provide meaningful data for the mean distribution. If too few microspheres are injected, the results can be due to a random distribution of the microspheres so that some areas which are perfused by the vessel receive no spheres. In addition, when too few spheres are injected the numbers of spheres in an area may not contain enough radioactive label to provide meaningful data. Buckberg et al (1971) demonstrated that when an organ or region contains over four hundred microspheres these criterion are satisfied.

By taking advantage of the different energy spectra of gamma-emitting nuclides, it is possible to differentiate between microspheres injected at different times or in different places (Heymann et al, 1977). In the studies on arterial distribution, at least two differently labelled fifteen micron microspheres were used. In some studies four different injections, and thus four differently labelled spheres were used. The nuclide labels used were ^{125}I, ^{141}Ce, ^{51}Cr, ^{85}Sr, and ^{95}Nb. Using a 512-channel multichannel pulse-height analyzer (Searle Analytic, Des Plaines, Illinois) the gamma-emitting spectrum of each isotope can be differentiated. This allows separate consideration of each artery's distribution in the same animal.

The microspheres were purchased from the Nuclear Products Division of the 3M Company. They were suspended in a 10% dextran solution with 0.19% polyoxyethylene 80 sorbitan monooleate to prevent aggregation of the spheres. Approximately one million microspheres were placed in a one ml. mixing chamber. Over a two minute period, five ml. of saline were flushed through the chamber into the artery. This resulted in almost all of the microspheres in the chamber being introduced into the artery.

At the completion of the experiment, the dog was sacrificed by giving a bolus intravenous injection of KCl. The brain was removed and fixed in formaldehyde. It was later dissected into pieces as shown in Figure 3 and Table I. The individual tissue pieces were carbonized in an oven and then analyzed for the amount of each nuclide which it contained. The data were analyzed by calculating the total counts found in the brain due to a specific nuclide. The results for each animal were calculated as the percent of the total counts in the brain that were found in each tissue piece and as the percent of the total counts in the brain that are found in each tissue piece divided by the tissue weight in grams. The values were expressed as the mean of the calculated values for each animal $\pm$ S.E.M. The weights of each dissected tissue piece were measured on the fixed tissue after dissection (Table I).

II.E. Statistical Methods

All results are expressed as the mean $\pm$ S.E.M. The significance of the cardiovascular and endocrine changes was determined using analysis

of variance for repeated measures (ANOVAR) (Winer, 1971) and Newman-Keuls multiple range testing (Zar, 1974). When possible, a two-way analysis of variance for repeated measures was used to analyze differences between treatment groups. If the control values were significantly different between groups this comparison is not permissible.

Table I

Weights of Dissected

Areas of Brain

area of brain	weight
1. right cerebellum	4.26 ± 0.12
2. left cerebellum	4.19 ± 0.11
3. right medulla	1.46 ± 0.06
4. left medulla	1.42 ± 0.04
5. right pons	1.04 ± 0.03
6. left pons	1.02 ± 0.03
7. right midbrain	1.11 ± 0.03
8. left midbrain	1.12 ± 0.03
9. right hypothalamus	0.86 ± 0.05
10. left hypothalamus	0.84 ± 0.03
11. right anterior cortex	8.45 ± 0.28
12. left anterior cortex	9.34 ± 0.31
13. right middle cortex	14.59 ± 0.45
14. left middle cortex	14.21 ± 0.38
15. right posterior cortex	9.25 ± 0.34
16. left posterior cortex	8.73 ± 0.22

Weights are expressed in grams as the mean ± S.E.M., n = 29.

Figure 3

Schematic representation of the areas separated in the dissection of the brain. The left and right sides were separated. Areas from the right side are labelled with odd numbers. Areas from the left side are labelled with even numbers. See Table I for further descriptions.

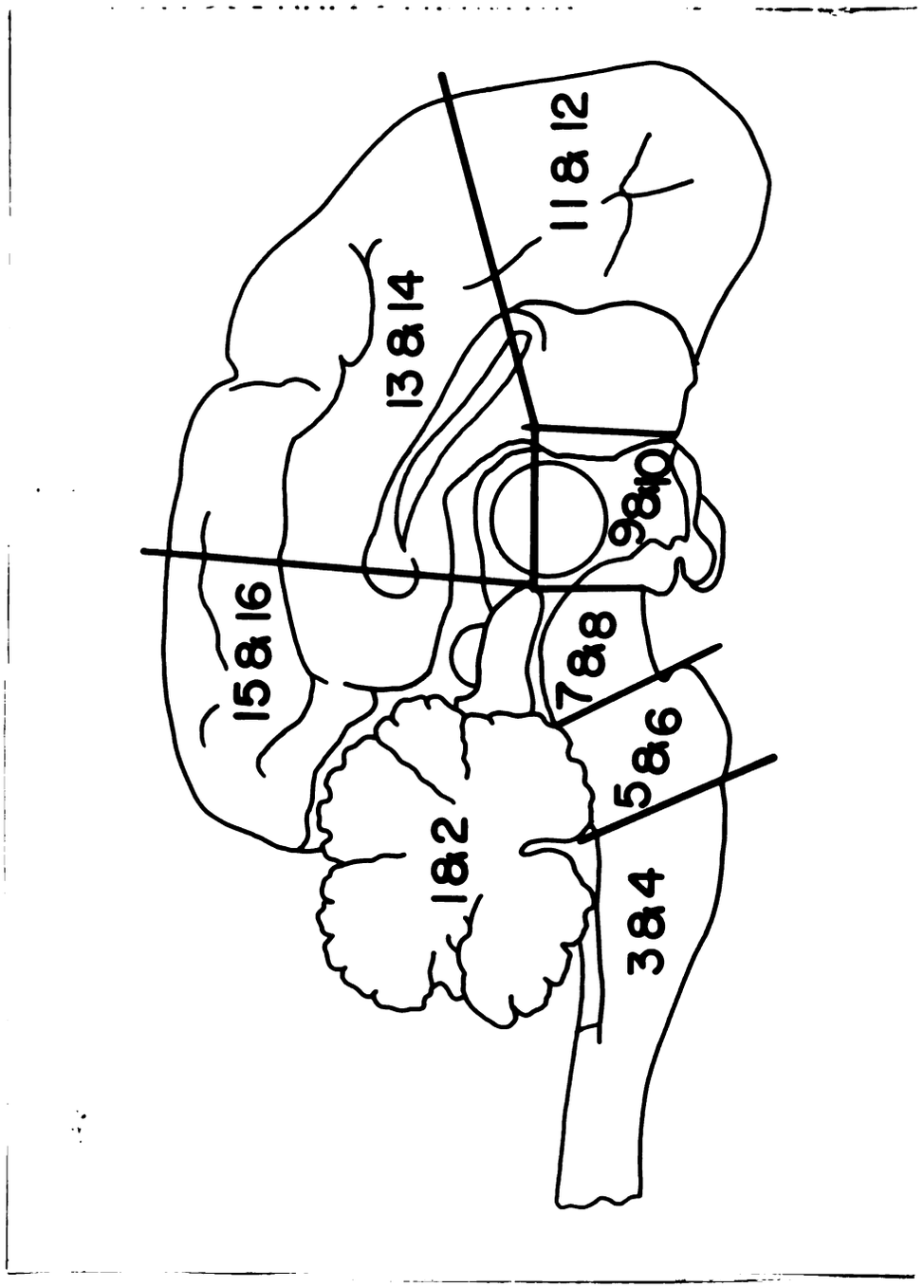

FIGURE 3

Section III

RESULTS

III-A. The Distribution of the Vertebral and Carotid Arteries

The distribution of microspheres injected at different vascular sites into the brains of dogs with catheters in both vertebral arteries and both carotid arteries is summarized in Tables II and III. Microspheres injected into the vertebral arteries primarily were found in caudal structures, however a significant proportion passed anteriorly into the middle and posterior cortex. Although most of the microspheres injected into the carotid artery were distributed in the cortex of the injected side, some microspheres passed caudally into the midbrain, pons and medulla. In these dogs, the hindbrain appeared to be primarily perfused by the vertebral arteries with some mixing with carotid blood. The hypothalamus received blood from all four arteries to the brain. The cerebral cortex received a mixed blood supply with the anterior cortex receiving more blood from the carotids than vertebrals. Compared to the anterior cortex the middle and posterior cortex received higher proportions of blood from the vertebral arteries.

The distribution of microspheres injected into the carotid arteries of dogs with catheters only in the carotid arteries is summarized in Tables IV and V. Virtually no microspheres were distributed to the cerebellum, midbrain, pons or medulla. The rostral structures in the brain were perfused by the carotid arteries. Although some microspheres were distributed to the side opposite to the injected side, most

microspheres were distributed to the side of the injected vessel. The relatively large numbers of microspheres which cross to the anterior cortex on the opposite through the anterior communicating artery.

The distribution of microspheres injected into dogs with catheters in both carotid arteries and with the basilar artery clipped is summarized in Tables VI and VII. In contrast to dogs without the basilar clipped, microspheres injected into the carotid arteries of these animals were found in the cerebellum, pons and midbrain. The number of microspheres per gram tissue in the pons and the cerebellum was relatively low compared to other brain regions which were perfused by the carotid arteries in this preparation. This suggests that normally, the entire pons and cerebellum are not perfused by the carotid arteries. It is probable that the cerebellum receives blood from the superior cerebellar artery, which is a branch of the rostral basilar artery. In all the regions receiving blood from the carotid arteries there were relatively more microspheres distributed to the injected side. This was similar to the dogs with only carotid artery catheters without the basilar artery clipped.

The distribution of microspheres injected into the vertebral arteries in dogs with the basilar artery clipped is summarized in Tables VIII and IX. Most of the microspheres were distributed in the pons, medulla and cerebellum. In most dogs no microspheres were found rostral to the pons, but in one animal some microspheres reached the midbrain. This was not surprising since in this animal the basilar

artery was clipped near the junction of the pons and the midbrain. In the same animal a very small number of microspheres were found in the right hypothalamus.

In two dogs, only the vertebral arteries were catheterized and microspheres were injected into the catheters. In one of these dogs too few spheres were injected to make any conclusions on the distribution of vertebral arteries. In the other dog the distribution of microspheres was similar to the distribution of microspheres injected into the vertebral arteries when the carotid arteries were catheterized.

Because clipping the basilar artery could interfere with the blood flow to the brain we investigated the effect of this procedure on regional blood flow in the brain. The data in Table X illustrates that clipping the basilar artery did not have any effects on the blood flow to any brain region.

III.B. The Cardiovascular Responses to Clonidine Infusion via Different Intravascular Routes

The changes in systolic blood pressure, diastolic blood pressure and heart rate in response to clonidine infusion via different intravascular routes are summarized in Tables XI, XII and XIII. Intravenous infusion of clonidine (2 ug/kg) consistently caused small decreases in systolic and diastolic blood pressure ($p < 0.001$) and decreased

heart rate ($p < 0.001$). These changes persisted for at least the 60 minutes until the experiment ended.

Infusion of clonidine into the vertebral arteries of dogs without the basilar artery clipped (a total dose of 2 ug/kg; 1 ug/kg into each vertebral artery) decreased systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), and heart rate ($p < 0.001$). The decreases in systolic and diastolic blood pressure were significantly greater than the decrease due to intravenous clonidine infusion ($p < 0.05$) even though the control values were somewhat lower. The heart rate response was not significantly different from the response to intravenous clonidine infusion.

Infusion of clonidine (2 ug/kg) into the carotid arteries of dogs without the basilar artery clipped caused decreases in systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), and heart rate ($p < 0.001$) which were not different from the changes after intravenous infusion of the same dose of clonidine.

Infusion of clonidine (2 ug/kg) into the vertebral arteries in dogs with the basilar artery clipped significantly decreased systolic ($p < 0.001$) and diastolic blood pressure ($p < 0.001$). Statistical comparison of this treatment with intravenous clonidine infusion was not possible using two-way ANOVAR since the control values were different ($p < 0.05$). However, two-way ANOVAR did not demonstrate any significant difference between clonidine infusion via the vertebral arteries in animals with or without the basilar artery clipped. Clonidine infusion into the vertebral arteries in dogs with the basilar artery clipped caused no significant change in

heart rate although there was a small decrease in heart rate. This lack of response was significantly different from the response to intravenous ($p < 0.05$) or intravertebral arterial ($p < 0.01$) infusion of clonidine.

Infusion of clonidine (2 ug/kg) into the carotid arteries in dogs with the basilar artery clipped decreased systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), and heart rate ($p < 0.001$). These responses were not statistically different from the response to intravenous clonidine infusion or from the response to clonidine infusion via the carotid arteries in dogs without the basilar artery clipped. It is worth noting that the heart rate returned to control values by 60 minutes in this series of experiments.

Infusion of a lower dose of clonidine (a total dose of 0.5 ug/kg; 0.25 ug/kg into each carotid artery) (Table XIX) into the carotid arteries of dogs without the basilar artery clipped consistently caused a small decrease in systolic blood pressure ($p < .01$) and diastolic blood pressure ($p < .05$). This treatment had no effect on heart rate. Subsequent infusion of the same dose of clonidine via the vertebral arteries caused further decreases in systolic ($p < 0.001$) and diastolic ($p < 0.001$) blood pressure. Infusion of clonidine into the vertebral arteries decreased the heart rate 15 ($p < 0.001$) and 30 minutes ($p < 0.01$) after the beginning of the infusion.

III.C. The Endocrine Response to Clonidine Infusion via Different Intravascular Routes

III-C.1. Changes in Plasma Renin Activity (PRA)

The changes in PRA resulting from clonidine infusion into different intravascular sites are summarized in Tables XIV and XV. Intravenous infusion of clonidine (2 ug/kg) had no effect on PRA. Clonidine (2 ug/kg) infusion into the vertebral arteries of dogs without the basilar artery clipped caused significant falls in PRA 30 minutes ($p < 0.001$) and 60 minutes ($p < 0.001$) after the infusion began. Clonidine (2 ug/kg) infusion into the carotid arteries of dogs without the basilar artery clipped decreased PRA 60 minutes after the infusion began ($p < 0.05$). If the results are expressed as the percent change from control levels, clonidine infusion via the vertebral arteries decreased PRA 30 ($p < 0.001$) and 60 minutes ($p < 0.001$) after its infusion and infusion of clonidine via the carotid arteries decreased PRA 30 ($p < 0.05$) and 60 minutes ($p < .01$) after its infusion.

In dogs with the basilar artery clipped, infusion of clonidine (2 ug/kg) into the vertebral arteries or into the carotid arteries had no effect on PRA. However, in two dogs with the basilar artery clipped intravenous administration of clonidine (30 ug/kg) resulted in decreases in PRA from 11 to 6 ng AI/ml/3hr and from 45 to 20 ng AI/ml/3hr. This indicates that clonidine can cause decreases in PRA in dogs with the basilar artery clipped.

Infusion of a lower dose of clonidine (0.5 ug/kg) into the carotid arteries did not change PRA. Subsequent infusion of the same dose of clonidine into the vertebral arteries also had no effect on PRA (see Table XIX).

III-C.2. Changes in Plasma ACTH Levels and In Plasma 11-Hydroxy-corticosteroid (Corticoid) Levels

The changes in plasma ACTH levels and in plasma corticoid levels resulting from the infusion of clonidine via different intravascular routes are summarized in Tables XVI and XVII. Intravenous clonidine infusion (2 ug/kg) had no effect on ACTH or 11-hydroxycorticosteroid plasma levels. Infusion of clonidine (2 ug/kg) into the vertebral arteries of dogs without the basilar artery clipped decreased ACTH levels 15 ($p < 0.01$) and 30 minutes ($p < 0.05$) after the start of the infusion. In these animals there was no change in corticoid levels. Infusion of clonidine (2 ug/kg) into the carotid arteries of dogs without the basilar artery clipped decreased plasma ACTH levels for at least the 60 minutes until the experiment ended ($p < 0.001$). In these animals there was also a reduction in corticoid levels ($p < 0.001$). Infusion of clonidine (2 ug/kg) into the vertebral arteries of dogs with the basilar artery clipped had no effect on ACTH or corticoid levels. Infusion of clonidine into the carotid arteries of dogs with the basilar artery clipped significantly decreased ACTH ($p < 0.001$) during the 60 minutes following infusion. Corticoid levels were decreased 15 minutes ($p < 0.05$) after this treatment.

Using a two-way ANOVA statistical comparisons of the ACTH responses to clonidine infusion into the carotid arteries and into the vertebral arteries was not possible because of the difference in control values. The plasma ACTH levels decreased more in dogs without the basilar artery clipped when clonidine was infused into the vertebral

artery than in dogs with the basilar artery clipped ($p < 0.05$). Plasma ACTH levels also decreased more in response to infusion of clonidine into the vertebral arteries of dogs without the basilar artery clipped than in response to intravenous clonidine infusion ($p < 0.05$). The ACTH response to intracarotid clonidine infusion was similar in dogs with or without the basilar artery clipped. There were no significant differences between the corticoid responses to clonidine infusion via different intravascular routes.

The infusion of lower doses of clonidine (0.5 ug/kg) into the carotid or vertebral arteries did not affect ACTH or 11-hydroxy-corticosteroid levels (Table XIX).

III.C.3. Changes in Plasma Growth Hormone (GH) Levels

The changes in plasma GH levels resulting from the infusion of clonidine via different intravascular routes are summarized in Table XVIII. Intravenous infusion of clonidine (2 ug/kg) had no effect. Infusion of clonidine (2 ug/kg) into the vertebral arteries of dogs without the basilar artery clipped increased GH levels 15 ($p < 0.01$) and 30 minutes ($p < 0.05$) after the infusion began. Infusion of clonidine (2 ug/kg) via the carotid arteries of dogs with the basilar artery clipped caused a similar increase in GH levels 15 ($p < 0.001$) and 30 minutes ($p < 0.01$) after the infusion began. Infusion of clonidine (2 ug/kg) via the vertebral arteries in dogs with the basilar artery clipped did not change plasma GH levels. Infusion of clonidine (2 ug/kg) into the carotid arteries of dogs with basilar

artery clips caused significant increases in plasma GH levels 15 ($p < 0.01$), 30 ($p < 0.01$) and 45 minutes ($p < 0.05$) after the infusion began. Statistical comparisons between treatments showed that there was no difference between the GH response to intravenous clonidine infusion and the infusion of clonidine via the vertebral arteries in dogs with the basilar artery clipped. The responses to infusion of clonidine via the vertebral or carotid arteries in dogs without the basilar artery clipped or via the carotid artery in dogs with the basilar artery clipped were not statistically different from each other. These treatments all resulted in greater increases in GH than intravenous infusion of clonidine ($p < 0.01$) or than infusion of clonidine via the vertebral arteries of dogs with the basilar artery clipped ($p < 0.01$).

There were no changes in plasma GH levels in response to the infusion of a lower dose of clonidine (0.5 ug/kg) into the carotid arteries or into the vertebral arteries (Table XIX).

Table II

Percent of Total Counts in Different Areas of the Brains of Dogs with Catheters in Both Carotid and Both Vertebral Arteries

area of brain	right vertebral artery	left vertebral artery	right carotid artery	left carotid artery
1. right cerebellum	24.7 ± 8.6	14.5 ± 4.0	11.6 ± 5.7	1.9 ± 1.3
2. left cerebellum	20.0 ± 7.5	21.2 ± 8.0	2.9 ± 1.0	3.4 ± 1.8
3. right medulla	9.1 ± 3.7	4.8 ± 1.2	2.1 ± 0.9	0.2 ± 0.2
4. left medulla	8.0 ± 3.7	4.0 ± 1.6	1.0 ± 0.3	0.2 ± 0.1
5. right pons	3.3 ± 0.9	2.8 ± 0.5	1.1 ± 0.4	0.3 ± 0.2
6. left pons	4.8 ± 1.4	4.9 ± 0.4	1.0 ± 0.3	0.4 ± 0.3
7. right midbrain	1.1 ± 0.8	1.9 ± 0.7	1.3 ± 0.5	0.4 ± 0.3
8. left midbrain	4.0 ± 2.3	5.3 ± 1.3	1.5 ± 0.9	1.5 ± 1.1

9.	right hypothalamus	0.8 ± 0.6	1.1 ± 0.6	1.8 ± 0.6	0.2 ± 0.1
10.	left hypothalamus	1.0 ± 0.8	1.4 ± 0.7	0.2 ± 0.1	1.4 ± 0.9
11.	right anterior cortex	0.1 ± 0.05	0.6 ± 0.3	11.1 ± 4.5	2.2 ± 2.1
12.	left anterior cortex	1.7 ± 1.0	2.4 ± 1.2	7.2 ± 2.3	19.3 ± 2.9
13.	right middle cortex	2.0 ± 1.8	3.7 ± 2.9	25.6 ± 5.5	9.0 ± 3.4
14.	left middle cortex	8.8 ± 4.1	12.7 ± 5.2	7.3 ± 1.5	37.0 ± 5.0
15.	right posterior cortex	2.4 ± 2.2	5.0 ± 4.1	17.8 ± 4.1	7.5 ± 4.7
16.	left posterior cortex	7.9 ± 4.5	11.1 ± 3.9	2.7 ± 0.9	0.6 ± 0.5
	TOTAL BRAIN	100	100	100	100

Both vertebral arteries and both carotid arteries were catheterized. Microspheres were injected into each artery. Results are expressed as the mean of the percent of the total counts per area of brain ± S.E.M., n = 4. In this and all subsequent tables the values are rounded off to the first decimal place.

Table III

Percent of Total Counts in Different Areas of the Brains of Dogs with Catheters in Both Carotid and Both Vertebral Arteries Divided by the Tissue Weight

	right vertebral artery	left vertebral artery	right carotid artery	left carotid artery
1. right cerebellum	3.9 ± 1.0	2.7 ± 0.7	0.8 ± 0.3	0.3 ± 0.2
2. left cerebellum	4.0 ± 1.5	4.4 ± 1.7	0.6 ± 0.2	0.6 ± 0.4
3. right medulla	6.1 ± 2.1	3.5 ± 0.9	1.5 ± 0.6	0.2 ± 0.2
4. left medulla	5.5 ± 2.7	4.5 ± 1.2	0.5 ± 0.2	0.2 ± 0.1
5. right pons	3.4 ± 1.2	2.6 ± 0.6	1.0 ± 0.4	0.2 ± 0.1
6. left pons	3.5 ± 1.0	3.9 ± 0.7	0.9 ± 0.4	0.3 ± 0.2
7. right midbrain	0.8 ± 0.6	1.3 ± 0.5	1.0 ± 0.3	0.3 ± 0.3
8. left midbrain	3.0 ± 1.8	4.1 ± 0.9	1.2 ± 0.8	0.7 ± 0.3

9.	right hypothalamus	0.8 ± 0.6	1.4 ± 0.9	2.2 ± 0.9	1.3 ± 0.6
10.	left hypothalamus	1.2 ± 0.7	1.4 ± 0.7	0.2 ± 0.1	0.2 ± 0.2
11.	right anterior cortex	0.0 ± 0.0	.05± 0.03	1.1 ± 0.5	1.0 ± 0.5
12.	left anterior cortex	0.5 ± 0.4	0.7 ± 0.6	1.0 ± 0.5	0.5 ± 0.2
13.	right middle cortex	0.1 ± 0.1	0.2 ± 0.1	1.3 ± 0.3	2.0 ± 0.2
14.	left middle cortex	0.5 ± 0.2	0.5 ± 0.4	0.4 ± 0.1	0.8 ± 0.5
15.	right posterior cortex	0.2 ± 0.2	0.5 ± 0.4	1.7 ± 0.4	0.8 ± 0.5
16.	left posterior cortex	0.8 ± 0.5	1.2 ± 0.5	0.3 ± 0.1	1.4 ± 0.3

Both vertebral arteries and both carotid arteries were catheterized. Microspheres were injected into each artery. Results are expressed as the mean of the percent of the total counts (Table II) divided by tissue weight in grams ± S.E.M., n = 4.

Table IV

Percent of Total Counts in Brain in Different Areas of the Brains of Dogs with Catheters in Both Carotid Arteries and No Catheters in the Vertebral Arteries

	area of brain	right carotid artery	left carotid artery
1.	right cerebellum	0 ± 0	0 ± 0
2.	left cerebellum	0 ± 0	0 ± 0
3.	right medulla	0 ± 0	0 ± 0
4.	left medulla	0 ± 0	0 ± 0
5.	right pons	0 ± 0	0 ± 0
6.	left pons	0 ± 0	0 ± 0
7.	right midbrain	0.1 ± 0.1	0 ± 0
8.	left midbrain	0.1 ± 0.1	0.1 ± 0.1
9.	right hypothalamus	0.9 ± 0.2	0 ± 0
10.	left hypothalamus	0.1 ± 0.1	0.6 ± 0.2
11.	right anterior cortex	31.3 ± 2.5	9.6 ± 1.9
12.	left anterior cortex	6.7 ± 2.0	28.2 ± 2.8
13.	right middle cortex	40.7 ± 4.4	7.2 ± 1.2
14.	left middle cortex	3.3 ± 1.1	34.5 ± 1.2
15.	right posterior cortex	18.5 ± 2.5	0.5 ± 0.1
16.	left posterior cortex	0.3 ± 0.2	19.2 ± 2.7
	TOTAL BRAIN	100	100

Both carotid arteries were catheterized. Microspheres were injected into each artery. Results are expressed as the mean of the percent of the total counts per area of brain ± S.E.M., n = 7.

Table V

Percent of Total Counts in Different Areas of the Brains of Dogs with Catheters in Both Carotid and Vertebral Arteries Divided by the Tissue Weight

area of brain	right carotid artery	left carotid artery
1. right cerebellum	0 ± 0	0 ± 0
2. left cerebellum	0 ± 0	0 ± 0
3. right medulla	0 ± 0	0 ± 0
4. left medulla	0 ± 0	0 ± 0
5. right pons	0 ± 0	0 ± 0
6. left pons	0 ± 0	0 ± 0
7. right midbrain	0.1 ± 0.1	0 ± 0
8. left midbrain	0 ± 0	0 ± 0
9. right hypothalamus	1.0 ± 0.2	0 ± 0
10. left hypothalamus	0.1 ± 0.1	0.8 ± 0.3
11. right anterior cortex	3.5 ± 0.4	1.0 ± 0.2
12. left anterior cortex	0.7 ± 0.3	2.8 ± 0.3
13. right middle cortex	3.0 ± 0.3	0.6 ± 0.1
14. left middle cortex	0.2 ± 0.1	2.7 ± 0.2
15. right posterior cortex	1.9 ± 0.2	0 ± 0
16. left posterior cortex	0 ± 0	2.2 ± 0.3

Both carotid arteries were catheterized. Microspheres were injected into each artery. Results are expressed as the mean of the percent of total counts (Table IV) divided by tissue weight in grams ± S.E.M., n=7.

Table VI

Percent of Total Counts in Brain in Different Areas of the Brains of Dogs with Catheters in Both Carotid Arteries and with the Basilar Artery Clipped

	area of brain	right carotid artery	left carotid artery
1.	right cerebellum	4.3 ± 0.9	4.0 ± 1.0
2.	left cerebellum	2.0 ± 1.0	5.0 ± 1.0
3.	right medulla	0 ± 0	0 ± 0
4.	left medulla	0 ± 0	0 ± 0
5.	right pons	0.7 ± 0.3	0.4 ± 0.2
6.	left pons	0.4 ± 0.2	1.0 ± 0.4
7.	right midbrain	3.0 ± 0.6	0.7 ± 0.1
8.	left midbrain	1.0 ± 0.5	2.0 ± 0.5
9.	right hypothalamus	2.7 ± 0.7	0.5 ± 0.2
10.	left hypothalamus	0.2 ± 0.1	1.5 ± 0.3
11.	right anterior cortex	18.3 ± 2.1	9.0 ± 2.0
12.	left anterior cortex	3.3 ± 2.1	20.0 ± 2.0
13.	right middle cortex	37.3 ± 3.4	8.0 ± 3.0
14.	left middle cortex	2.8 ± 1.0	30.0 ± 1.0
15.	right posterior cortex	22.7 ± 2.6	2.0 ± 1.0
16.	left posterior cortex	1.0 ± 0.6	18.0 ± 3.0
	TOTAL BRAIN	100	100

Both carotid arteries were catheterized and the basilar artery was clipped at the mid-pontine level. Microspheres were injected into each artery. Results are expressed as the mean of the percent of the total counts per area of brain ± S.E.M., n = 6.

Table VII

Percent of Total Counts in Brain in Different Areas of the Brains of Dogs with Catheters in Both Carotid Arteries and with the Basilar Artery Clipped Divided by the Tissue Weight

		right carotid artery	left carotid artery
1.	right cerebellum	1.0 ± 0.2	0.7 ± 0.2
2.	left cerebellum	0.5 ± 0.2	1.4 ± 0.2
3.	right medulla	0 ± 0	0 ± 0
4.	left medulla	0 ± 0	0 ± 0
5.	right pons	0.6 ± 0.3	0.3 ± 0.2
6.	left pons	0.4 ± 0.2	0.8 ± 0.3
7.	right midbrain	2.6 ± 0.4	0.6 ± 0.1
8.	left midbrain	0.8 ± 0.4	2.3 ± 0.2
9.	right hypothalamus	2.9 ± 0.6	0.6 ± 0.2
10.	left hypothalamus	0.2 ± 0.1	1.6 ± 0.3
11.	right anterior cortex	2.1 ± 0.3	1.0 ± 0.3
12.	left anterior cortex	0.3 ± 0.2	2.1 ± 0.1
13.	right middle cortex	2.5 ± 0.2	0.5 ± 0.2
14.	left middle cortex	0.2 ± 0.1	2.0 ± 0.1
15.	right posterior cortex	2.8 ± 0.3	0.2 ± 0.1
16.	left posterior cortex	0.1 ± 0.1	2.0 ± 0.3

Both carotid arteries were catheterized and the basilar artery was clipped at the mid-pontine level. Microspheres were injected in each artery. Results are expressed as the mean of the percent of the total counts (Table VI) divided by tissue weight in grams ± S.E.M., n=6.

Table VIII

Percent of Total Counts in Brain in Different Areas of the Brains of Dogs with Catheters in Both Vertebral Arteries and with the Basilar Artery Clipped

	area of brain	right vertebral artery	left vertebral artery
1.	right cerebellum	33.0 ± 4.0	28.0 ± 3.0
2.	left cerebellum	24.0 ± 5.0	31.0 ± 3.0
3.	right medulla	14.0 ± 2.0	13.0 ± 1.0
4.	left medulla	12.0 ± 2.0	15.0 ± 2.0
5.	right pons	9.0 ± 2.0	8.0 ± 1.0
6.	left pons	6.0 ± 1.0	5.0 ± 1.0
7.	right midbrain	0.5 ± 0.3	0.5 ± 0.3
8.	left midbrain	0.5 ± 0.4	0.5 ± 0.3
9.	right hypothalamus	0.1 ± 0.1	0 ± 0
10.	left hypothalamus	0 ± 0	0 ± 0
11.	right anterior cortex	0 ± 0	0 ± 0
12.	left anterior cortex	0 ± 0	0 ± 0
13.	right middle cortex	0 ± 0	0 ± 0
14.	left middle cortex	0 ± 0	0 ± 0
15.	right posterior cortex	0 ± 0	0 ± 0
16.	left posterior cortex	0 ± 0	0 ± 0
	TOTAL BRAIN	100	100

Both vertebral arteries were catheterized and the basilar artery was clipped at the mid-pontine level. Microspheres were injected into each artery. Results are expressed as the mean of the percent of the total counts per area of brain ± S.E.M., n = 6.

Table IX

Percent of Total Counts in Brain in Different Areas of the Brains of Dogs with Catheters in Both Vertebral Arteries and with the Basilar Artery Clipped Divided by the Tissue Weight

	area of brain	right vertebral artery	left vertebral artery
1.	right cerebellum	7.0 ± 3.0	7.0 ± 1.0
2.	left cerebellum	5.0 ± 2.0	7.0 ± 1.0
3.	right medulla	13.0 ± 1.0	10.0 ± 1.0
4.	left medulla	11.0 ± 2.0	12.0 ± 1.0
5.	right pons	10.0 ± 1.0	8.0 ± 1.0
6.	left pons	6.0 ± 1.0	5.0 ± 1.0
7.	right midbrain	0.5 ± 0.3	0.5 ± 0.3
8.	left midbrain	0.5 ± 0.3	0.5 ± 0.3
9.	right hypothalamus	0.1 ± 0.1	0 ± 0
10.	left hypothalamus	0 ± 0	0 ± 0
11.	right anterior cortex	0 ± 0	0 ± 0
12.	left anterior cortex	0 ± 0	0 ± 0
13.	right middle cortex	0 ± 0	0 ± 0
14.	left middle cortex	0 ± 0	0 ± 0
15.	right posterior cortex	0 ± 0	0 ± 0
16.	left posterior cortex	0 ± 0	0 ± 0

Both vertebral arteries were catheterized and the basilar artery was clipped at the mid-pontine level. Microspheres were injected into each artery. Results are expressed as the mean of the percent of the total counts per area of brain ± S.E.M., n = 6.

Table X

The Effect of Clipping the Basilar Artery on Regional Blood Flows

area of brain	injection #1	injection #2	basilar artery clipped ↓ injection #3	injection #4
1. right cerebellum	86 ± 2	79 ± 5	80 ± 8	69 ± 7
2. left cerebellum	83 ± 6	73 ±11	73 ± 7	76 ± 8
3. right medulla	57 ± 7	67 ± 8	58 ± 6	44 ± 6
4. left medulla	50 ± 4	67 ± 9	54 ± 4	46 ± 9
5. right pons	43 ± 1	57 ± 5	44 ± 8	38 ± 5
6. left pons	40 ± 2	58 ± 4	57 ± 7	49 ±12
7. right midbrain	84 ± 4	79 ± 4	72 ±11	82 ±14
8. left midbrain	78 ± 5	80 ± 6	75 ± 4	65 ± 3
9. right hypothalamus	65 ± 4	66 ± 5	57 ± 8	49 ± 9
10. left hypothalamus	62 ± 3	63 ± 5	72 ± 8	42 ±10
11. right anterior cortex	71 ± 3	67 ± 3	65 ± 7	64 ± 4
12. left anterior cortex	73 ± 4	68 ± 2	67 ± 4	71 ± 4
13. right middle cortex	67 ± 6	64 ± 6	62 ± 5	67 ± 7
14. left middle cortex	67 ± 3	64 ± 3	62 ± 3	69 ± 4
15. right posterior cortex	72 ± 5	69 ± 7	65 ± 3	66 ± 2
16. left posterior cortex	72 ± 4	70 ± 4	66 ± 3	64 ± 1

Microspheres with different nuclide labels were injected into the left ventricle and flows were calculated using a reference blood sample taken during four different injections of microspheres. Between the second and third injection the basilar artery was clipped at the mid-pontine level. Results are expressed as the flow per 100 grams to the area of brain in ml. ± S.E.M., n = 4.

Table XI

Systolic Blood Pressure Responses to Clonidine Infusion via Different Intravascular Routes

time (min.)	-20	0	15	30	45	60
Intravenous n = 7	156 ± 5	155 ± 5	146*** ± 4	146*** ± 4	147*** ± 3	148*** ± 4
Intravertebral Artery n = 8	139 ± 8	140 ±11	107*** ± 5	110*** ± 5	114*** ± 7	121*** ± 8
Intracarotid Artery n = 9	165 ± 7	164 ± 6	152*** ± 7	153*** ± 6	154*** ± 7	155*** ± 6
Intravertebral Artery with the Basilar Artery Clipped; n = 7	133 ± 8	138 ± 8	116*** ± 5	116*** ± 5	120*** ± 7	119*** ± 6
Intracarotid Artery with the Basilar Artery Clipped; n = 6	144 ± 5	145 ± 6	139*** ± 6	138** ± 7	138*** ± 6	138*** ± 6

Systolic blood pressure was recorded continuously throughout the experiment. Values are expressed as the mean ± S.E.M. (torr). Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ug/kg into each side) was infused from 0 to 2 minutes via the carotid or vertebral arteries.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table XII

Diastolic Blood Pressure Responses to Clonidine Infusion via Different Intravascular Routes

time (min.)	-20	0	15	30	45	60
Intravenous n = 7	116 ± 4	116 ± 4	108*** ± 2	108*** ± 2	109*** ± 3	109** ± 2
Intravertebral Artery n = 8	104 ± 6	106 ± 6	74*** ± 5	77*** ± 5	78*** ± 5	84*** ± 6
Intracarotid Artery n = 9	102 ±13	101 ±13	89*** ±12	90*** ±12	92*** ±12	93*** ±12
Intravertebral Artery with the Basilar Artery Clipped; n = 7	94 ± 7	95 ± 8	76*** ± 5	75** ± 5	76*** ± 6	76*** ± 6
Intracarotid Artery with the Basilar Artery Clipped; n = 6	109 ± 4	108 ± 6	105** ± 4	102*** ± 4	102*** ± 4	102*** ± 4

Diastolic blood pressure was recorded continuously throughout the experiment. Values are expressed as the mean ± S.E.M. (torr). Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ug/kg into each side) was infused from 0 to 2 minutes via the carotid or vertebral arteries.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table XIII

Heart Rate Response to Clonidine Infusion

via Different Intravascular Routes

time (min.)	-20	0	15	30	45	60
Intravenous n = 7	123 ± 9	126 ± 8	106*** ± 8	105*** ± 9	110** ±10	115** ±11
Intravertebral Artery n = 8	139 ±12	139 ±11	112*** ±12	111*** ±14	117*** ±15	123** ±15
Intracarotid Artery n = 9	120 ± 8	122 ± 7	103*** ± 6	103*** ± 7	105*** ± 7	107*** ± 6
Intravertebral Artery with the Basilar Artery Clipped; n = 7	137 ±12	139 ±11	132 ±14	130 ±14	132 ±15	134 ±15
Intracarotid Artery with the Basilar Artery Clipped; n = 6	127 ±11	128 ±11	113*** ±13	113*** ±11	119*** ±11	126 ±13

Heart rate values are expressed as the mean ± S.E.M. (beats/minute). Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ug/kg into each side) was infused from 0 to 2 minutes via the carotid or vertebral arteries.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table XIV

Changes in Plasma Renin Activity in Response to Clonidine Infusion via Different Intravascular Routes

time (min.)	-20	0	15	30	45	60
Intravenous n = 7	45 ±10	44 ± 9	45 ± 9	43 ± 9	-	40 ± 7
Intravertebral Artery n = 8	47 ± 9	45 ± 7	42 ± 6	34*** ± 6	-	29*** ± 6
Intracarotid Artery n = 9	52 ±13	50 ±12	51 ±15	44 ±13	-	38* ±10
Intravertebral Artery with the Basilar Artery Clipped; n = 6	37 ±11	40 ±12	37 ±13	38 ±14	38 ±12	41 ±15
Intracarotid Artery with the Basilar Artery Clipped; n = 6	28 ± 5	28 ± 6	30 ± 6	30 ± 6	31 ± 8	34 ± 9

Values are expressed as the mean ± S.E.M. (ng angiotensin I generated/ml/3hr). Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ng/kg into each side) was infused from 0 to 2 minutes via the carotid or vertebral arteries.

* $p < 0.05$

** $0 < 0.01$

*** $p < 0.001$

Table XV

Changes in Plasma Renin Activity (expressed as Δ% control values) in Response to Clonidine Infusion via Different Intravascular Routes

time (min.)	0	15	30	45	60
Intravenous n = 7	+ 4 ± 6	+ 4 ± 6	+ 3 ±12	-	- 1 ±10
Intravertebral Artery n = 8	0 ± 7	- 8 ± 7	-28*** ± 4	-	-40** ± 5
Intracarotid Artery n = 9	0 ± 3	+ 3 ±14	-14* ±12	-	-33** ± 6
Intravertebral Artery with the Basilar Artery Clipped; n = 6	+ 5 ± 5	-15 ±12	- 7 ±11	- 6 ±12	- 3 ±12
Intracarotid Artery with the Basilar Artery Clipped; n = 6	- 2 ± 7	0 ± 7	2 ±10	3 ±11	+16 ±21

Values are expressed as the mean of the percent change from the control value at - 20 minutes ± S.E.M. Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ug/kg into each side was infused from 0 to 2 minutes via the carotid or vertebral artery.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table XVI

Changes in Plasma ACTH Levels in Response to Clonidine Infusion via Different Intravascular Routes

time (min.)	-20	0	15	30	45	60
Intravenous n = 7	76 ± 8	80 ± 6	63 ± 7	74 ±12	-	72 ± 4
Intravertebral Artery n = 8	79 ±23	76 ±20	56** ±15	57* ±15	-	70 ±20
Intracarotid Artery n = 9	102 ±23	104 ±25	64*** ±17	64*** ±22	-	67*** ±22
Intravertebral Artery with the Basilar Artery Clipped; n = 7	80 ± 9	85 ±10	74 ±16	85 ±14	100 ±19	99 ±26
Intracarotid Artery with the Basilar Artery Clipped; n = 6	94 ±29	101 ±31	46*** ±17	58*** ±16	72* ±24	65** ±22

ACTH values are expressed as the mean ± S.E.M. (pg/ml). Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ug/kg into each side) was infused from 0 to 2 minutes via the carotid or vertebral arteries.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table XVII

Changes in Plasma 11-Hydroxycorticosteroids in Response to Clonidine Infusion via Different Intravascular Routes

time (min.)	-20	0	15	30	45	60
Intravenous n = 7	7.6 ±1.1	7.9 ±1.1	7.0 ±1.1	7.5 ±1.2	7.4 ±1.4	8.1 ±1.3
Intravertebral Artery n = 8	7.4 ±0.7	7.7 ±0.7	6.9 ±0.9	6.8 ±1.0	-	7.5 ±0.7
Intracarotid Artery n = 9	8.0 ±1.0	8.1 ±1.1	6.3** ±1.1	5.3*** ±0.9	-	6.9* ±1.2
Intravertebral Artery with the Basilar Artery Clipped; n = 7	9.1 ±0.8	9.4 ±0.8	9.4 ±1.0	9.5 ±1.0	9.9 ±0.8	10.4 ±0.7
Intracarotid Artery with the Basilar Artery Clipped; n = 6	10.0 ±1.0	10.1 ±1.3	7.9* ±1.4	8.3 ±1.5	8.2 ±1.4	8.5 ±1.5

11-Hydroxycorticosteroid values are expressed as the mean ± S.E.M. (ug/dl). Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ug/kg into each side) was infused from 0 to 2 minutes via the carotid or vertebral arteries.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table XVIII

Changes in Plasma GH Levels in Response to Clonidine Infusion via Different Intravascular Routes

time (min.)	-20	0	15	30	45	60
Intravenous n = 7	2.1 ±0.4	2.3 ±0.5	3.1 ±0.5	2.6 ±0.4	-	2.6 ±0.4
Intravertebral Artery n = 6	3.4 ±1.4	3.6 ±1.5	9.1** ±3.1	6.6* ±2.4	-	2.6 ±0.9
Intracarotid Artery n = 9	2.9 ±0.6	3.0 ±0.6	14.6*** ±3.5	10.6** ±2.4	-	4.7 ±0.8
Intravertebral Artery with the Basilar Artery Clipped; n = 7	2.1 ±0.3	2.1 ±0.3	2.2 ±0.4	2.3 ±0.4	2.5 ±0.5	2.5 ±0.4
Intracarotid Artery with the Basilar Artery Clipped; n = 6	2.8 ±0.4	2.9 ±0.4	8.0** ±1.2	8.3** ±2.6	7.3* ±3.6	3.5 ±0.8

Values are expressed as the mean ± S.E.M. (ng/ml). Clonidine (2 ug/kg) was infused from 0 to 2 minutes via the femoral vein. Clonidine (1 ug/kg into each side was infused from 0 to 2 minutes via the carotid or vertebral arteries.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Table XIX

Changes in Plasma Hormone Levels, Blood Pressure and Heart Rate in Response to Clonidine Infusion into the Carotid Arteries Followed by Clonidine Infusion into the Vertebral Arteries

time (min.)	-20	0	15	30	60	75	90	120
		0.25 ug/kg clonidine injected into each carotid artery ↓			0.25 ug/kg clonidine injected into each vertebral artery ↓			
Systolic Blood Pressure (torr)	170 ± 13	171 ± 12	162** ±11	163** ±14	163** ±13	128*** ±14	129*** ±13	142** ±10
Diastolic Blood Pressure (torr)	127 ± 8	129 ± 9	120* ± 7	121* ± 8	121* ± 9	89*** ±11	93** ±11	105* ± 9
Heart Rate (beats/min.)	137 ± 13	145 ± 12	134 ±13	135 ±13	142 ±12	111*** ± 7	120** ± 8	133 ±10

Plasma Renin Activity (ng AI generated/ml/hr)	45 ± 12	51 ± 10	43 ±11	41 ±14	55 ± 9	42 ± 9	47 ±11	80 ±16
ACTH (pg/ml)	78 ± 19	97 ± 21	83 ±27	88 ±25	99 ±32	102 ±36	107 ±30	102 ±27
11-Hydroxycorticosteroids (ng/dl)	9.9 ± 1.8	9.9 ± 1.9	9.0 ±2.3	9.0 ±1.9	10.0 ±2.7	10.9 ±1.9	11.1 ±1.8	11.6 ±2.6
GH (ng/ml)	1.3 ± 0.4	1.3 ± 0.5	2.6 ±1.0	1.2 ±0.5	1.7 ±0.6	2.0 ±0.6	2.5 ±1.3	2.8 ±1.3

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n = 5 values are expressed as the mean ± S.E.M. Clonidine (.25 ug/kg into each side) was infused from 0 to 2 minutes via the carotid arteries. Clonidine (.25 ug/kg into each side) was infused from 60 to 62 minutes via the vertebral arteries.

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

Section IV

DISCUSSION OF RESULTS

IV.A. The Distribution of the Vertebral and Carotid Arteries

The studies of the distribution of the vertebral and carotid arteries were initiated in order to determine where clonidine injected into the vertebral or carotid arterial catheters of dogs could be exerting its actions. Radionuclide labelled microspheres were injected into the catheters and their distribution in the brain was used as an indicator of the regions injected clonidine would reach.

Our results on the distribution of the vertebral and carotid arteries in the dog essentially confirm the results of Wellens et al (1975). When the vertebral and carotid arteries were catheterized by either their technique or our technique, the hypothalamus was perfused by blood from both the vertebral and carotid arteries. Regions rostral to the hypothalamus were primarily perfused by blood from the carotid arteries although some vertebral arterial blood appeared to reach these areas. Regions caudal to the hypothalamus were primarily perfused by blood from the vertebral arteries although the carotid artery also contributed to the supply of blood to these regions. Wellens et al (1975) discriminated between the distribution of blood to the anterior and posterior hypothalamic regions. They found that the posterior hypothalamus received its blood supply almost exclusively

from the vertebral arteries whereas the anterior hypothalamus received a mixture of carotid and vertebral arterial blood. We did not divide the hypothalamus into anterior and posterior regions.

In our studies if the carotid arteries were catheterized without vertebral arterial catheterization, the carotid arteries did not supply the hindbrain. This could be the normal pattern of distribution, or the carotid could normally supply the hindbrain and catheterization of the arteries could cause a decrease in the area if perfuses. Since the vertebral arteries are smaller than the carotid arteries, it is more likely that the catheterization of the vertebral arteries interferes with flow through the artery. When the vertebral arteries are catheterized in dogs without the basilar artery clipped the carotid arteries may provide blood to the hindbrain. In dogs with the vertebral arteries totally occluded the carotid arteries supply blood to the entire hindbrain (Gildenberg and Ferrario, 1977).

Gildenberg and Ferrario (1977) used radio-autographic techniques to demonstrate that in dogs with the basilar artery clipped, the regions perfused by the carotid arteries and vertebral arteries change. We confirmed these results using the microsphere method.

As discussed above, in dogs without the basilar artery clipped the vertebral arteries supply most of the blood to the midbrain and regions caudal to the midbrain. After clipping the basilar artery the vertebral arteries no longer supply blood to any regions rostral to the midbrain. In most animals, the vertebral

artery only perfuses the medullary, pontine and cerebellar regions after clipping the basilar artery. The carotid arteries perfuse the rest of the brain and also partially perfuse the cerebellum.

Conclusions about the site of action of drugs injected via different intravascular routes can be made in light of these distribution results. By injecting drugs into the carotid or vertebral arteries after clipping the basilar artery we can determine if their actions are exerted caudal or rostral to the midpontine level. By injecting drugs into the carotid arteries when the vertebral arteries are not cannulated we can determine if the drug's actions are exerted rostral to the midbrain. If a drug exerts its actions only when it is injected into the vertebral arteries it is likely that it acts in the hindbrain, hypothalamus or posterior cortical regions since other regions are perfused by both the carotid and the vertebral arteries.

It is important that clipping the basilar artery has no effects on blood flow to any brain region. These results suggest that the clipping procedure does not seriously alter brain function by causing lesions or ischemia of any particular region.

IV.B. The Site of Action of Clonidine on Blood Pressure, Heart Rate, ACTH, GH and PRA

The experiments described in the previous sections were designed to determine the sites of action of clonidine. Conclusions about the site of action of clonidine on the cardiovascular and endocrine systems can be made by considering the responses to the infusion of clonidine via

different intravascular routes in light of the distribution results.

Sattler and Van Zweiten (1967) demonstrated that infusion of clonidine via the vertebral artery in pentobarbital anesthetized dogs decreases blood pressure at doses which are either ineffective or have small effects when infused intravenously. Katic et al (1972) made similar observations in chloralase anesthetized dogs and also claimed that infusion of clonidine into the carotid arteries had a slight depressor effect. These results were confirmed in the present studies in pentobarbital anesthetized dogs. Although the 2 ug/kg dose of clonidine infused caused significant falls in blood pressure when given intravenously, the hypotensive response was significantly greater when the clonidine was infused into the vertebral artery. Infusion into the carotid arteries caused falls which were not different from intravenous treatment. Infusion of a lower dose of clonidine (0.5 ug/kg) into the carotid arteries also caused a small decrease in blood pressure; however, subsequent infusion of the same dose into the vertebral arteries caused a greater decrease in blood pressure. These results indicate that the hypotensive effect of clonidine is due to its action on a site or sites in the area perfused by the vertebral arteries.

In dogs with the basilar artery clipped, infusion of clonidine via the vertebral arteries caused marked decreases in blood pressure whereas infusion via the carotid arteries had a depressor effect which was greater than the response to intravenous infusion of clonidine. These results further demonstrate that the primary site at which clonidine elicits its hypotensive effects is in the medulla or pons. Evidence from other

experiments leads to similar conclusions. Large lesions of the ventral medulla prevent the hypotensive response to clonidine (Laubie and Schmitt, 1977). Furthermore, infusion of clonidine directly into the motor nuclei of the vagus or into the medullary reticular formation decreased blood pressure (Sinha et al, 1975).

The finding that the infusion of phenoxybenzamine into the fourth cerebral ventricle but not the third cerebral ventricle prevents the hypotensive response to intravenous clonidine infusion (Ganong, 1977) also indicates that clonidine is acting in the hindbrain.

Intravenous infusion of clonidine (2 ug/kg) decreased heart rate. Similar decreases in heart rate resulted from clonidine infusion into the vertebral or carotid arteries of dogs without the basilar artery clipped and via the carotid arteries of dogs with the basilar artery clipped. It is difficult to explain the lack of effect on heart rate of clonidine infusion into the vertebral arteries in dogs with the basilar artery clipped. Since there was no significant decrease in heart rate following the infusion of clonidine into the vertebral arteries, it would be premature to make conclusions based on these studies without repeating the experiments. However, Laubie (1976a) showed that clonidine acts at different sites to affect baroreceptor reflexes and to produce decreases in heart rate and blood pressure. It is possible that clonidine could be affecting heart rate in different manners at a variety of sites. Clonidine infused into the vertebral arteries of dogs with the basilar artery clipped may act on a site at which clonidine causes increases in heart rate. This action may be more

pronounced with infusions into the vertebral arteries of dogs with the basilar artery clipped than with infusions via other intravascular routes.

Infusion of clonidine into the vertebral arteries or the carotid arteries decreased PRA. Intravenous infusion of the same dose of clonidine had no effect on PRA. Combined with the findings that administration of clonidine into the cisterna magna (Onesti et al, 1971) or into the third cerebral ventricle (Reid et al, 1975) decrease PRA, these observations suggest that intravenous infusion of higher doses of clonidine act in the central nervous system to decrease PRA.

The results of the blood flow distribution studies show that in dogs with only the carotid arteries catheterized and no clip on the basilar artery, the carotid arteries do not perfuse the hindbrain. These results therefore show that clonidine can act in rostral parts of the brain to lower PRA. Since the vertebral and carotid arteries both perfuse the hypothalamic region and the posterior cortical region, the results suggest that clonidine acts in these regions to lower PRA.

In dogs with the basilar artery clipped, infusion of clonidine via the vertebral or carotid arteries had no effect on PRA. The injection of microspheres into the left atrium showed that clipping the basilar artery did not cause ischemia of any region of the brain, so that the lack of clonidine's effect on PRA in dogs with the basilar artery clipped cannot be due to destruction of its site of action. In two dogs, intravenous infusion of a high dose of clonidine after clipping the basilar artery decreased PRA. This also demonstrates that the site of action was intact. One possible explanation of these results

is that clonidine lowers PRA by acting on a site perfused by the vertebral and carotid arteries. The flow through the vertebral arteries is approximately one third of the flow through the common carotid arteries (personal observation). Infusion of a given dose via the vertebral arteries may be more effective since the concentration of clonidine in the blood reaching this hypothetical area is higher after vertebral arterial infusion than after carotid arterial infusion. After clipping the basilar artery, the area is perfused solely by the carotid arteries. The PRA lowering effect of clonidine infusion into the carotid arteries in dogs without the basilar artery clipped was not highly significant. It is possible that the added surgical stress of clipping the basilar artery increased PRA. This added stress could have prevented PRA from decreasing to the same extent when clonidine was infused into the carotid arteries of dogs with the basilar artery clipped compared to the decrease in dogs without the basilar artery clipped.

Ganong et al (1978) demonstrated that intravenous clonidine infusion does not lower PRA in dogs with spinal cord transections, however in dogs with the midbrain transected clonidine does decrease PRA (Ganong, W.F., unpublished results). These experiments show that clonidine decreases PRA by acting in regions caudal to the brain transection. Consideration of the differential effects of intravascular clonidine infusion suggest that clonidine acts in the rostral portion of the hindbrain or posterior hypothalamic region to decrease PRA. These regions could be perfused by both the vertebral and carotid arteries. The midbrain transection experiments of Ganong et al

are difficult to explain if this is the only site of action. It is possible that clonidine is acting at sites in the hypothalamus (Ueda et al, 1967) and in sites in the hindbrain (Passo et al, 1971; Richardson et al, 1974) which have been shown to alter PRA.

Plasma ACTH levels were decreased by the infusion of low doses of clonidine into the vertebral or carotid arteries. Plasma 11-hydroxy-corticosteroid (corticoid) levels were decreased only by the infusion of clonidine via the carotid artery. Intravenous infusion of the same dose of clonidine had no effect on plasma ACTH or corticoid levels. Clonidine infusion via the vertebral arteries in dogs with the basilar artery clipped did not effect ACTH or corticoid levels. However, clonidine infusion via the carotid arteries in dogs with the basilar artery clipped did decrease ACTH and corticoid levels. These results show that clonidine acts in an area normally perfused by both the carotid and vertebral arteries. The results of the distribution studies show that this area is probably in the hypothalamic or posterior cortical brain regions. Only the carotid arteries perfuse these areas when the basilar artery is clipped.

Ganong (1977) demonstrated that infusion of phenoxybenzamine into the third cerebral ventricle but not into the fourth cerebral ventricle prevented the fall in corticoid levels caused by clonidine. These results together with the results of injecting clonidine into different intravascular sites show that clonidine acts in the rostral portions of the brain to decrease ACTH release. Jones et al (1976) demonstrated that in the isolated rat hypothalamus norepinephrine

decreases CRF release. It is possible that clonidine decreases ACTH release in the dog by acting on adrenergic receptors in the hypothalamus.

Infusion of low doses of clonidine (2 ug/kg) into the vertebral and carotid arteries increased plasma GH levels. Intravenous infusion of the same dose had no effect. In dogs with the basilar artery clipped infusion of clonidine via the carotid arteries increased plasma GH levels, whereas infusion of clonidine via the vertebral arteries had no effect. These responses indicate that clonidine acts at sites in similar regions of the brain to increase GH levels and to decrease ACTH levels.

The GH response to intravascular infusion of clonidine appears to be greater than the response to intracerebroventricular infusion of clonidine. When relatively high doses of clonidine (5 ug/kg) were infused into the third cerebral ventricle GH levels were not increased (Lovinger et al, 1976). When the same dose was infused into the fourth cerebral ventricle GH was stimulated (Ganong, W.F., unpublished results). These results suggest that clonidine acts in the hindbrain to stimulate GH secretion. However, infusion of phenoxybenzamine into the third cerebral ventricle prevented the increase in GH levels in response to intravenous clonidine infusion (Ganong et al, 1977). Phenoxybenzamine infusion into the fourth cerebral ventricle had no effect on the GH response to intravenous clonidine.

The results of infusing clonidine into different intravascular sites and of the experiments infusing phenoxybenzamine into the third

or fourth ventricle both indicate that clonidine acts to increase GH secretion at a site in rostral regions of the brain. The results of intravascular infusions suggest that clonidine exerts this action in the hypothalamic or posterior cortical regions. There is no obvious explanation for the conflicting results from experiments infusing clonidine into the third or fourth cerebral ventricle.

Although the primary aim of these experiments was to determine the central site(s) of action of clonidine, the studies were initiated in order to gain greater insight into the central adrenergic system and its interaction with the cardiovascular and endocrine systems. Since clonidine appears to act on central receptors similar to the receptor which norepinephrine and epinephrine act upon (see section I.C.) and since clonidine crosses the blood-brain barrier it was the most appropriate agent for use in these studies.

Axons containing norepinephrine and epinephrine originate in the hindbrain and are distributed to sites throughout the brain (see section I.D.). Clonidine could be acting at one of these sites to initiate a series of cardiovascular and endocrine responses or it could be acting at several sites, in a manner similar to the endogenous central catecholamines. Hauesler (1973; 1974a) originally suggested that clonidine's actions on the cardiovascular system were due to its stimulation of adrenergic receptors in the nucleus tractus solitarius which are normally stimulated by baroreceptor fibers. He noted the similarity between the cardiovascular response to carotid sinus nerve stimulation and to clonidine. Although it appears that this explanation

is not totally satisfactory since clonidine still has blood pressure and heart rate lowering effects in animals with lesions of the nucleus tractus solitarius (Laubie and Schmitt, 1977) it is interesting that the mimicking of baroreceptor stimulation would also be expected to decrease PRA (Bunag et al, 1966), plasma ACTH levels (Gann, 1966) and plasma vasopressin levels (Share and Levy, 1962). It is also interesting that the endocrine and cardiovascular responses to clonidine are opposite to the responses to surgical stress which increases ACTH levels (Ganong et al, 1976), vasopressin levels (Moran et al, 1964), blood pressure and heart rate (Ganong, 1977c). In rats (Martin, 1976) but not in dogs (Lovinger et al, 1974) surgical stress decreases GH levels.

The results of the experiments described in this dissertation demonstrate that clonidine acts at different sites in the central nervous system to cause its responses. Clonidine acts in rostral portions of the brain to alter ACTH and GH. It acts in the hindbrain to decrease blood pressure. The blood pressure and heart rate results indicate that clonidine may be acting at separate sites to alter heart rate and blood pressure. A separate site of action also appears to mediate the PRA lowering effects of clonidine. Thus these results suggest that clonidine acts at various sites throughout the brain. It is likely that it is acting at the endings of monoamine containing neurons and therefore its effects provide insight into the role of the noradrenergic and/or adrenergic neurons in the brain. This is more likely than the possibility that clonidine stimulates an integrated response to a specific type of sensory input by acting at one site.

Whether or not these neurons have essential functions in terms of the response to stress or possibly in the adaption to stressful stimuli remains to be elucidated.

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