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Prostaglandin production by sympathetic postganglionic neurons and its regulation by noradrenaline

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PROSTAGLANDIN PRODUCTION BY SYMPATHETIC POSTGANGLIONIC NEURONS AND ITS REGULATION BY NORADRENALINE

by

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TABLE OF CONTENTS

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FIGURES

TABLES

ABSTRACT

Prostaglandin E_2 and prostacyclin (prostaglandin I_2) modulate adrenergic neurotransmission, and contribute to a sympathetically-dependent hyperalgesia and plasma extravasation. The source of the prostaglandins mediating these actions is, however, not known. We have evaluated whether sympathetic postganglionic neurons synthesize these prostaglandins, whether production of prostaglandins by these neurons can contribute to sensitization of primary afferent nociceptors, and how this production is regulated.

To examine the contribution of sympathetic postganglionic nerve terminals in prostaglandin mediated hyperalgesia, we studied the effect of arachidonic acid (the precursor to prostaglandins) on the nociceptive threshold in the rat. Intradermal injection of arachidonic acid but not linoleic acid, in the rat hindpaw, decreased the mechanical nociceptive threshold. This hyperalgesic effect was prevented by indomethacin, an inhibitor of prostaglandin synthesis, or by prior surgical removal of the lumbar sympathetic chain. These findings suggest that sympathetic postganglionic nerve terminals can metabolize arachidonic acid to hyperalgesic prostaglandins.

To test the hypothesis that sympathetic postganglionic nerve terminals are a source of prostaglandins, we measured production of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ (the stable metabolite of prostacyclin) by homogenates of adult rat superior cervical ganglia. These homogenates produced significant amounts of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$. Prostaglandin production was markedly attenuated by neonatal administration of 6-hydroxydopamine, which selectively destroys sympathetic postganglionic neurons, supporting the hypothesis that sympathetic postganglionic neurons synthesize prostaglandin E_2 and I_2.

Regulation of sympathetic postganglionic neuronal production of prostaglandins was examined by studying the effect of noradrenaline, which is known to act at presynaptic receptors on sympathetic nerve terminals and to stimulate prostaglandin production in a

variety of non-neuronal cells. Administration of noradrenaline increased, in a dose-dependent manner, the levels of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$ synthesized by homogenates of superior cervical ganglia. This noradrenaline-induced prostaglandin production, as well as basal production, was abolished by selective destruction of adrenergic sympathetic postganglionic neurons in the ganglia using 6-hydroxydopamine. Conversely, elimination of preganglionic cholinergic sympathetic nerve terminals in the ganglia had no effect on prostaglandin synthesis. Mepacrine (a phospholipase inhibitor) and indomethacin (a cyclooxygenase inhibitor) attenuated both basal and noradrenaline-stimulated prostaglandin production. To evaluate the receptor at which noradrenaline acts to stimulate prostaglandin production, we studied the effects of selective alpha-adrenergic receptor antagonists. Yohimbine ($alpha_2$-selective), but not prazosin ($alpha_1$-selective), suppressed the noradrenaline dose-response curve for prostaglandin production. These findings provide evidence to support the suggestion that noradrenaline stimulates *de novo* synthesis of prostaglandin E_2 and I_2, and that this action is mediated by an $alpha_2$-adrenergic receptor on the sympathetic postganglionic neuron.

In conclusion, we have demonstrated that sympathetic postganglionic neurons synthesize prostaglandin E_2 and I_2, and that this production is stimulated by noradrenaline acting on an $alpha_2$-adrenergic receptor. These studies support the hypothesis that the prostaglandins which modulate sympathetic neurotransmission and mediate sympathetically-dependent hyperalgesia and plasma extravasation are generated in response to noradrenaline acting on a presynaptic $alpha_2$-adrenergic receptor.

INTRODUCTION

Prostaglandins (PGs) are 20 carbon fatty acids first described by von Euler in the 1930s (von Euler, 1934-36). The synthesis of PGs, which occurs in practically all mammalian cells, requires the release of arachidonic acid from membrane phospholipid pools. Arachidonic acid can then be metabolized by either the lipoxygenase pathways yielding products such as leukotrienes, dihydroxyeicosatetraenoic acids (diHETEs) and lipoxins, or metabolized by the cyclooxygenase pathway generating PGs of the E, I, D, or F series, or thromboxanes. Since their initial discovery, PGs have been found to contribute to numerous physiological functions. Their involvement in thrombogenesis (O'Brien, 1968; Vargaftig, 1973; Bunting, 1976; Moncada, 1988), inflammation [i.e. vasodilation (Berström, 1959; Williams, 1973; Moncada, 1979), edema (plasma extravasation) (Williams, 1973; Wedmore, 1981; Basran, 1982), and hyperalgesia (Ferreira, 1972; 1973; Davies, 1984)], and the pathogenesis of fever (Dinarello, 1978) is well characterized.

Prostaglandins are also intimately involved in several aspects of sympathetic nervous system function (Hedqvist, 1976). They are released by many organs following sympathetic nerve stimulation or catecholamine administration, including spleen (Davies, 1968), heart (Wennmalm, 1971; Junstad, 1973), kidney (Davis, 1972; Needleman, 1974), gastrointestinal tract (Coceani, 1967), and vas deferens (Hedqvist, 1974). There is substantial evidence that E-type and I-type PGs contribute to feedback inhibition of neurotransmitter release by an action on sympathetic postganglionic neuron (SPGN) terminals (Hedqvist, 1972; Hedqvist, 1974; Frame, 1975), as well as at preganglionic cholinergic nerve terminals (Dun, 1980; Belluzzi, 1982); an effect which appears to be mediated through a decrease in calcium conductance (Mo, 1985).

More recently, it has been proposed that PGs mediate sympathetically-dependent hyperalgesia and plasma extravasation. The hyperalgesic properties of PGE_2 and I_2 are well known (Kuhn, 1973; Ferreira, 1978). For example, bradykinin and noradrenaline

(NA) can elicit a hyperalgesia that is blocked by cyclooxygenase inhibitors, as well as by sympathectomy (Lembeck, 1976; Levine, 1986). Prostaglandins generate hyperalgesia by binding specific receptors on small diameter afferent nerve fibers to decrease the nociceptive threshold, and potentiate the actions of other inflammatory mediators. Similarly, plasma extravasation induced by factors that activate sympathetic nerve terminals in the knee joint of the rat is attenuated by indomethacin and by sympathectomy (Coderre, 1989). This indomethacin-induced inhibition of sympathetically-dependent plasma extravasation can be reversed by injection of PGE_2 during activation of SPGN terminals.

The specific source(s) of the PGs regulating neuroeffector function and mediating sympathetically-dependent hyperalgesia and plasma extravasation are not known. There is evidence that postsynaptic cells generate PGs following sympathetic nerve stimulation (Gilmore, et al., 1968; Junstad and Wennmalm, 1973; Jeremy, et al., 1985; Pipili and Poyser, 1987). However, there is also support for a presynaptic source of PGs (Stjarne, 1972; Levine, et al., 1986). In fact, Webb and colleagues have reported that rat superior cervical ganglia (SCG) generate E-type PGs (Webb, et al., 1978), and others have shown that portal vein and vas deferens depend on sympathetic nerve terminals for PG production (Greenberg, 1978; Petkov and Radomirov, 1980).

The goals of these studies are to test the hypothesis that SPGNs are capable of synthesizing PGE_2 and PGI_2, and that this production is enhanced by a physiological stimulus.

MATERIALS AND METHODS

Materials

Arachidonic and linoleic acid, (-)-arterenol bitartrate ([-]-noradrenaline), Krebs-Ringer bicarbonate buffer, indomethacin, mepacrine, prazosin hydrochloride, and yohimbine hydrochloride were purchased from Sigma Chemical Corp., St. Louis, Missouri. 6-hydroxydopamine hydrobromide (6-OHDA) was purchased from Aldrich Chemical Corp., Milwaukee, Wisconsin. Materials used in the radioimmunoassays included ^{3}H-PGE_2 and ^{3}H-6-keto-$PGF_{1\alpha}$ purchased from New England Nuclear Co., Boston, Massachusetts, and anti-PGE_2, anti-6-keto-$PGF_{1\alpha}$, PGE_2 and 6-keto-$PGF_{1\alpha}$ purchased from Advanced Magnetics (Seragen), Boston, Massachusetts.

Mechanical Nociceptive Threshold

The mechanical nociceptive flexion reflex was quantified in 250-300 g male Sprague-Dawley rats (Bantin and Kingman, Fremont, California) using the Randall-Selitto paw withdrawal test (Randall and Selitto, 1957). The baseline nociceptive threshold is defined as the weight, in grams, at which the rat withdraws its paw. In normal rats, the average baseline threshold was 104.25 g $\pm$ 6.01 (n=8). Hyperalgesia is defined as a significant decrease in the nociceptive threshold produced by a test compound. After measuring the baseline threshold for paw-withdrawal, we injected arachidonic acid intradermally in a volume of 2.5 µl of saline. The nociceptive threshold was remeasured 20 min after injection of arachidonic acid. The effect of intradermal arachidonic acid was studied in normal rats, in rats sympathectomized by surgical ablation of the lumbar sympathetic chain 1 week prior to further experiments, and, to rule out a direct effect of arachidonic acid on primary afferents, in rats that received the cyclooxygenase inhibitor indomethacin (4 mg/kg, i.p.) 30 min prior to injection of arachidonic acid. Indomethacin was dissolved in a vehicle of 2% sodium bicarbonate and then titrated to pH 7.2 with monosodium phosphate. To evaluate nonspecific effects of unsaturated fatty acids, intradermal injection of linoleic acid (an 18-

carbon dienoic fatty acid with similar detergent properties to arachidonic acid) was also tested.

Superior Cervical Ganglion Homogenates

Superior cervical ganglia, harvested under pentobarbital anesthesia (65 mg/kg, i.p.), from pairs of adult male and female Sprague-Dawley rats (Bantin & Kingman, Fremont, CA), were desheathed in Krebs-Ringer bicarbonate buffer (1.80 g/l glucose; 0.10 g/l magnesium chloride/$6H_20$; 0.34 g/l potassium chloride; 7.00 g/l sodium chloride; 0.10 g/l sodium phosphate dibasic (anhydrous); 0.18 g/l sodium phosphate monobasic (anhydrous)) to which was also added 0.20 g/l ascorbic acid, 1.26 g/l sodium bicarbonate, and 0.28 g/l calcium chloride, then titrated to pH 7.40 at 37°C. Eight ganglia in 1.0 ml buffer were homogenized on ice using a glass tissue grinder (model #885000-0002, Kontes, Vineland, NJ). The homogenate was aliquoted into 250 µl samples, and following the addition of noradrenaline, antagonist or vehicle in 10 µl volumes, incubated in a 37°C shaking water bath for 30 min. Samples were briefly bubbled (2-3 sec) with $95\%O_2/5\%CO_2$ at the beginning of the incubation, and again after 15 min. Samples were then spun in a microfuge for 1 min to pellet membranes, and the supernatant removed and stored at -80°C for subsequent determination of PG concentration.

Chemical Sympathectomy

To produce a selective and permanent depletion of neurons in the SCG, some rats received 6-hydroxydopamine (6-OHDA), 150 mg/g subcutaneously, on alternate days, for two weeks, starting on neonatal day 2 (Angeletti and Levi-Montalcini, 1970).

Surgical Denervation

To destroy sympathetic preganglionic nerve terminals in the SCG, a 5 mm segment of the preganglionic nerve trunk was surgically excised from 4-5 week old rats, and SCG harvested 2 weeks later. This procedure has been shown to reduce SCG choline acetyltransferase (a marker for the cholinergic preganglionic nerve terminals) by >90% (Quik, et al., 1986). A surgical-control (sham) group of rats were used in which the

preganglionic nerve trunk was exposed, but not cut, after which the animals recovered for two weeks before harvesting the SCG.

Phospholipase and Cyclooxygenase Inhibition

Phospholipase inhibition was performed by incubating SCG homogenates in the presence of mepacrine (1 mM). To assess the contribution of the cyclooxygenase pathway to PG production, whole SCGs were incubated in Krebs buffer with indomethacin (20 μM) (dissolved in a vehicle of 2% sodium bicarbonate and titrated to pH 7.2 with sodium monophosphate), or vehicle, for 30 min at 37°C. SCG homogenates were then prepared, and the experiment continued as outlined above.

Radioimmunoassay

The levels of PGE_2 in supernatants were measured by a radioimmunoassay that detects 10 pg/0.10 ml of PGE_2 (Goldyne et al., 1973). The levels of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) were determined by the same method using specific antisera to 6-keto-$PGF_{1\alpha}$,and this antisera could also detect 10 pg/0.01 ml. Each sample (100 μl) was assayed in duplicate, and the PG concentration calculated from the average.

Histology

Sections of SCG from normal and 6-OHDA-pretreated rats were also compared histologically. Ganglia were desheathed, fixed in 4% paraformaldehyde, 4% sucrose in 0.1 M phosphate buffer for 2 h, placed in 30% sucrose in buffer overnight, and then cut on a cryostat into 25 μm-thick sections and Wright -stained.

Statistical Analysis

Statistical analyses on changes in nociceptive thresholds in response to various treatments were performed using repeated measures analysis of variance (ANOVA). Prostaglandin levels in normal and 6-OHDA-pretreated ganglia were compared using an independent t-test. Statistical comparisons of the effects of various stimuli and inhibitors on PG production were performed using ANOVA and, when appropriate, the Newman-Keuls post-hoc test.

RESULTS

Intradermal injection of arachidonic acid produced a dose-dependent decrease in mechanical nociceptive threshold as indicated by a significant main effect of dose of arachidonic acid ($p<0.01$, two-way ANOVA) (Fig. 1). The effect of arachidonic acid was antagonized by indomethacin treatment or by prior surgical removal of the lumbar sympathetic chain. Specifically, the dose dependence of the effect of arachidonic acid on nociceptive threshold was abolished for these two groups (indicated by a significant main effect of treatment, $p<0.01$, two-way ANOVA). None of the tested doses of linoleic acid produced a significant change in nociceptive threshold. Indomethacin alone (i.e. without arachidonic acid) had no significant effect on nociceptive threshold (data not shown).

To test the hypothesis that SPGNs are the source of PGs in SPGN-dependent hyperalgesia, we measured PG production in normal and 6-OHDA-pretreated rats. On gross examination, SCG from rats pretreated with 6-OHDA, when compared with normal ganglia, were smaller in size, and when viewed by light microscopy, exhibited a selective depletion of the large-diameter cells in the ganglia (Fig. 2).

Prostaglandin E_2 and 6-keto-$PGF_{1\alpha}$ levels in SCG prior to homogenization were below detectable limits of the radioimmunoassays. Superior cervical ganglion homogenates from untreated rats produced significant levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ (Table 1). The amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$ were, however, significantly decreased in homogenates of SCG from 6-OHDA-pretreated rats, when compared to normal rats ($p<0.01$ for both PGE_2 and 6-keto-$PGF_{1\alpha}$). In fact, PGE_2 was undetectable and PGI_2 fell to 16% of the level obtained in control (untreated) rats.

Since sympathetic nerve stimulation and catecholamine administration increase PGs in organ perfusates (Coceani, 1967; Davies, 1968; Wennmalm, 1971; Davis, 1972; Junstad, 1973; Hedqvist, 1974; Needleman, 1974), we tested the ability of noradrenaline to stimulate PGE_2 and PGI_2 production by SCG homogenates. Noradrenaline (50 μM to 1

mM) increased, in a dose-dependent fashion, the levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in SCG homogenates (both $p<0.001$) (Fig. 3).

Both basal and NA-induced PG production were markedly diminished in rats that were pretreated neonatally with 6-OHDA (both $p<0.01$) (Fig. 4a,b). Previous denervation of the SCG by lesion of the preganglionic nerve trunk, however, failed to significantly attenuate the stimulatory effect of NA on PGE_2 (Fig. 4a) and 6-keto-$PGF_{1\alpha}$ (Fig. 4b) levels (both $p>0.05$).

Noradrenaline-induced PGE_2 and 6-keto-$PGF_{1\alpha}$ production was decreased by mepacrine, a phospholipase inhibitor (both $p<0.01$). Preincubation of SCG with indomethacin, at a dose (20 μM) which has been reported to be relatively selective for inhibition of cyclooxygenase (Somova, 1973; Flower, 1974; Willis, et al., 1972), also decreased NA-stimulated PG production (both $p<0.01$) (Fig. 5a,b).

To determine the adrenergic receptor at which NA acts to induce PG production, the effect of yohimbine (a selective $alpha_2$-adrenergic receptor antagonist) and prazosin (a selective $alpha_1$-adrenergic receptor antagonist) on the NA dose-response curve were measured. At concentrations approximating $10xK_i$, yohimbine significantly attenuated the dose response curve for NA-induced PGE_2 synthesis ($p<0.01$), whereas prazosin did not significantly affect the NA dose response curve ($p>0.05$) (Fig. 6). At the EC_{50} dose range for NA, the same dose of yohimbine inhibited approximately 40% of PGE_2 production ($p<0.01$), whereas prazosin did not significantly affect the NA-induced production of PGE_2 ($p>0.05$). At higher concentrations of NA, there was no significant antagonism by yohimbine or prazosin ($p>0.05$).

DISCUSSION

In these studies, intradermal injection of arachidonic acid produced a dose-dependent hyperalgesia. The observation that sympathectomy prevents this response demonstrates that arachidonic acid-induced hyperalgesia is dependent on SPGN terminals. Pretreatment with indomethacin also prevents arachidonic acid hyperalgesia, suggesting that arachidonic acid-induced hyperalgesia is mediated through a product of the cyclooxygenase pathway of arachidonic acid metabolism. Although use of indomethacin does not exclude the possibility that lipoxygenase products might also contribute to the arachidonic acid-induced hyperalgesia, the dose of indomethacin used in this study inhibits predominantly the cyclooxygenase pathway of arachidonic acid metabolism (Willis, 1972; Somova, 1973; Flower, 1974). In contrast to arachidonic acid injection, the injection of linoleic acid, a desaturated fatty acid similar in chain length to arachidonic acid, did not produce hyperalgesia. Failure of linoleic acid to produce hyperalgesia suggests that the indomethacin-reversible hyperalgesia induced by arachidonic acid injection is not the result of a nonspecific effect of fatty acids on endogenous arachidonic acid metabolism, but rather that arachidonic acid itself is being converted to PGs.

To determine whether SPGNs are a source of hyperalgesic PGs, we measured PGE_2 and 6-keto-$PGF_{1\alpha}$ production by SCG homogenates. We observed that the homogenates produced significant amounts of these two eicosanoids, both of which elicit hyperalgesia when injected into the skin (Ferreira, 1978). Importantly, treatment of neonatal rats with 6-OHDA, a selective toxin for catecholaminergic neurons, abolished detectable PGE_2 generation and markedly reduced 6-keto-$PGF_{1\alpha}$ production by adult SCG homogenates. Histological examination of SCG from rats treated with 6-OHDA confirmed the findings of others that pretreatment of rats, neonatally, with 6-OHDA depletes SPGNs (Angeletti, 1970). These findings support the hypothesis that SPGNs are capable of generating hyperalgesic PGs. The finding of residual 6-keto-$PGF_{1\alpha}$ production by SCG homogenates from rats pretreated with 6-OHDA might be due either to the small number of

SPGNs that remain in 6-OHDA pretreated ganglia, or to the production of 6-keto-$PGF_{1\alpha}$ by non-neuronal cells. The observation that in normal ganglia 6-keto-$PGF_{1\alpha}$ production is less than that of PGE_2 suggests that the residual 6-keto-$PGF_{1\alpha}$ in ganglia from 6-OHDA-pretreated rats is non-neuronal in origin.

To evaluate the ability of NA, the principle neurotransmitter of SPGNs, to stimulate PG production we studied its effect on PG production by SCG homogenates. Noradrenaline dose-dependently enhances the production of PGE_2 and 6-keto-$PGF_{1\alpha}$ by SCG homogenates, and this effect of NA is abolished by prior destruction of SPGNs with 6-OHDA. In contrast, surgical ablation of the preganglionic nerve trunk, which destroys the preganglionic nerve terminals in the SCG, did not diminish the increased eicosanoid production in response to NA. That 6-OHDA abolishes NA-stimulated PG production by SCGs strongly supports the hypothesis that NA can act on SPGNs to enhance the production of PGs. Furthermore, since the phospholipase inhibitor mepacrine and the cyclooxygenase inhibitor indomethacin both decrease NA-stimulated PG production, NA appears to stimulate *de novo* synthesis of PGs.

In evaluating the contribution of specific alpha-adrenergic receptors on SPGNs to NA-induced PG synthesis by SCG homogenates, we studied the effect of selective alpha-adrenergic receptor antagonists on NA-induced synthesis of PGE_2--the prostanoid with the larger response to NA stimulation. The $alpha_2$-adrenergic receptor antagonist yohimbine attenuated NA-induced PG synthesis, whereas the $alpha_1$-adrenergic receptor antagonist prazosin did not significantly affect NA-induced PG production. Loss of the inhibition of NA-induced PGE_2 production at the same dose of yohimbine, in the presence of higher doses of NA, demonstrates that the $alpha_2$ block exerted is a competitive antagonism and is compatible, therefore, with the suggestion that a receptor-mediated effect is involved. Importantly, the approximated K_i for yohimbine in our studies was 78 nM, well within ranges of K_i reported by other investigators (Starke, 1981; Goldberg and Robertson, 1983; Taniguchi et al., 1988). Although NA preferentially acts at alpha-adrenergic receptors, at

higher concentrations NA may also bind beta-receptors as well. However, even equimolar concentrations of the beta-adrenergic antagonists metoprolol ($beta_1$-selective) and butoxamine ($beta_2$-selective) failed to affect NA (1 mM) stimulation of PGE_2 synthesis (unpublished observations). Taken together, these results suggest that NA stimulates PG production by an action at a site with characteristics of the $alpha_2$-adrenergic receptor.

A relatively high concentration of NA was required to stimulate PG production by SCG homogenates, compared to the NA concentration usually required for similar receptor mediated actions in cultured cells. The concentration of NA in the incubation medium, however, does not necessarily reflect the concentration of NA at the cell membrane receptor. For example, extracellular enzymes present in the homogenates (e.g. catechol-o-methyl transferase) avidly metabolize catecholamines. In fact, Libet and colleagues, in electrophysiological studies using perfused SCG, required specific inhibitors of catechol-o-methyl transferase in order to observe a response to exogenous catecholamines (Ashe and Libet, 1979; Ashe and Libet, 1981; Mochida, et al., 1987). Of note, previous studies of NA-induced PG production by SCG have required doses in the range we employed (Webb, et al., 1978). To test the hypothesis, under our conditions, that a lower concentration of NA actually exists at the receptor level, we tested inhibition of NA-induced PGE_2 synthesis by receptor antagonists at considerably lower concentrations. Since these antagonists are not metabolized by enzymes that metabolize catecholamines, their concentration in the media will more precisely reflect their concentration at the receptor. We found that a low concentration of yohimbine (200 nM) attenuates NA-induced PGE_2 production, whereas prazosin (10 nM) has no effect. More importantly, the calculated K_i for yohimbine was well within the range reported in the literature (Starke, 1981, Goldberg and Robertson, 1983; Taniguchi et al., 1988), supporting the conclusion that this action of NA at the $alpha_2$-receptor is a physiologic effect.

Besides NA-induced hyperalgesia, the SPGN may be a source of PGs generated in response to other inflammatory mediators. For example, the plasma extravasation in the rat

knee joint induced by substance P, histamine and bradykinin is reduced by sympathectomy or indomethacin pretreatment, and restored by exogenous PGE_2 (Coderre et. al., 1989). Our data supports the suggestion that SPGN-derived PGE_2 can contribute to sympathetically-dependent plasma extravasation.

Noradrenaline appears to stimulate only PGI_2 synthesis in nerve injury-associated hyperalgesia (Taiwo and Levine, 1989), therefore, it was unexpected that NA stimulated both PGE_2 and PGI_2 synthesis in SPGN homogenates. One possible explanation is that SCG homogenization might destroy an existing segregation of the PGE_2 and PGI_2 enzymatic pathway components so that the common precursor of PGE_2 and PGI_2 (endoperoxide), generated by NA receptor activation, is now exposed to both PGI_2 synthase and PGE_2 isomerase. Alternatively, our findings may reflect a difference between neuronal terminals and cell bodies. Studies using whole ganglia or cultured SPGNs may be useful in testing these alternative explanations.

In summary, these studies demonstrate that SPGNs synthesize PGE_2 and PGI_2, the PGs that have been shown to function as modulators of adrenergic neurotransmission and effectors of sympathetically-dependent hyperalgesia and plasma extravasation. These studies also provide strong evidence that NA stimulates SPGNs to increase the synthesis of PGE_2 and PGI_2. Although in a variety of systems NA appears to bind a postsynaptic $alpha_1$-receptor to stimulate PG production (Gilmore, et al., 1968; Junstad and Wennmalm, 1973; Pipeli and Poyser, 1987; Trachte, 1987), our data suggests that a presynaptic action at an $alpha_2$ site on the SPGN terminal can also lead to PG synthesis. This suggests that the feedback inhibition of neurotransmitter release by $alpha_2$ receptors may result not only from receptor mediated changes in cyclic-AMP, but also as a result of PG actions on the presynaptic terminal.

A clinical correlate of this work applies to the observation that sympathetically maintained pain syndromes can be exacerbated by catecholamines (Wiesenfeld-Hallin, 1984). Our finding that SPGN-derived PGs contribute to sympathetically dependent

hyperalgesia in the rat suggests that, under these circumstances, NA acts on sympathetic nerve terminals to release PGs. This suggestion is consistent with our finding that NA-enhanced synthesis of PGs by SPGNs occurs, at least in part, via an action at a site with characteristics of the alpha$_2$-adrenergic receptor.

FIGURE LEGENDS

Figure 1. Effect of arachidonic acid and linoleic acid on mechanical nociceptive threshold in the hindpaw of the rat

Arachidonic acid (n=8) produces a dose-dependent decrease ($p<0.001$), whereas linoleic acid (n=8) has no detectable effect ($p<0.001$), on nociceptive threshold. Indomethacin (4 mg/kg), given intraperitoneally 30 min prior to arachidonic acid (n=6), prevents arachidonic acid-induced hyperalgesia at all doses up to 10 μg ($p<0.001$). Lumbar sympathectomy (n=12) also prevented hyperalgesia at all doses of arachidonic acid tested ($p<0.001$). Values and error bars are means ± SEM.

Figure 2. Effect of 6-hydroxydopamine on sympathetic postganglionic neurons in superior cervical ganglia.

Wright-stained 25-μm sections of untreated (**A**) and 6-hydroxydopamine-pretreated (**B**) superior cervical ganglia from adult rats demonstrate that 6-hydroxydopamine pretreatment produces a marked decrease in sympathetic postganglionic neurons. Calibration bar represents 30 μm.

Figure 3. The effect of noradrenaline on prostaglandin production by superior cervical ganglion homogenates.

Noradrenaline elicits a dose-dependent increase in PGE_2 (n=4) and 6-keto-$PGF_{1\alpha}$ (n=4) production by SCG homogenates from 4-5 week old adult rats incubated at 37°C for 30 min ($p<0.01$). Values and error bars in figure are means ± SEM.

Figure 4a,b. Effect of 6-hydroxydopamine and preganglionic denervation on noradrenaline-induced prostaglandin production by superior cervical ganglion homogenates.

SCG homogenates from 6-hydroxydopamine (6-OHDA) pretreated rats (n=4) show marked attenuation of baseline and noradrenaline stimulated (6-OHDA + NA, n=4) production of PGE_2 and 6-keto-$PGF_{1\alpha}$, when compared with levels in homogenates from

age-matched control (CON) and control plus noradrenaline (CON + NA) animals ($p<0.01$ for all comparisons). SCG homogenates from animals which had their SCG decentralized by lesion of the preganglionic nerve trunk (LES) 2 weeks prior to removal of SCG show the same level of noradrenaline stimulated PGE_2 and 6-keto-$PGF_{1\alpha}$ production (LES + NA, n=5) compared to age-matched (CON + NA, n=5) and sham surgery (SHAM + NA, n=4) controls ($p>0.05$ for all comparisons).

SCG used in the denervation experiments were from 6-7 week old rats, since we allowed an additional 2 weeks for complete degeneration of preganglionic nerve terminals. SCG from older animals generated greater PGE_2 production in response to NA (1 mM) stimulation than SCG from their 4-5 week old counterparts in figure 3 ($p<0.01$), whereas there was no significant difference between basal production. Conversely, SCG from 6-7 week old animals showed a marked increase in basal 6-keto-$PGF_{1\alpha}$ production while the percent increase in 6-keto-$PGF_{1\alpha}$ production in response to NA (1 mM) was less than from 4-5 week old animals (33% vs. 50%). All noradrenaline concentrations are 1 mM. Values and error bars in figure are means $\pm$ SEM.

Figure 5a,b. Effect of mepacrine and indomethacin on noradrenaline-induced prostaglandin production by superior cervical ganglion homogenates.

Mepacrine (MEP + NA, n=6) (1 mM) attenuates the stimulatory effect of noradrenaline (NA) (1 mM) on PGE_2 and 6-keto-$PGF_{1\alpha}$ production by SCG homogenates ($p<0.01$). SCG treated with indomethacin (INDO + NA, n=4) (20 μM) starting 30 min prior to homogenization, followed by addition of noradrenaline, also produce significantly less PGE_2 and 6-keto-$PGF_{1\alpha}$ than noradrenaline-treated vehicle controls (CON + NA, n=4) ($p<0.01$). All noradrenaline concentrations are 1 mM. Values and error bars in figure are means $\pm$ SEM. Control and NA-induced prostaglandin production were measured in SCG from 4-5 week old animals.

Figure 6. Effect of yohimbine and prazosin on noradrenaline-induced prostaglandin E_2 production by superior cervical ganglion homogenates.

The alpha$_2$-adrenergic receptor antagonist yohimbine (200 nM) suppressed the dose response curve for NA-induced PGE_2 production, whereas the alpha$_1$-adrenergic receptor antagonist prazosin (10 nM) had no effect on NA-induced PGE_2 production (two-way ANOVA: yohimbine vs. NA, $F=17.171$, p-value<0.01; prazosin vs. NA, $F=0.003$, $p=0.95$). There was a significant effect of yohimbine ($p=0.05$), but not prazosin, on basal PGE_2 production. K_i (Yohimbine) = 78 nM, using EC_{50} (Noradrenaline) = 4.22 ng/ml. Values and error bars in figure represent means $\pm$ SEM, and n=4 for all groups.

FIGURE 1

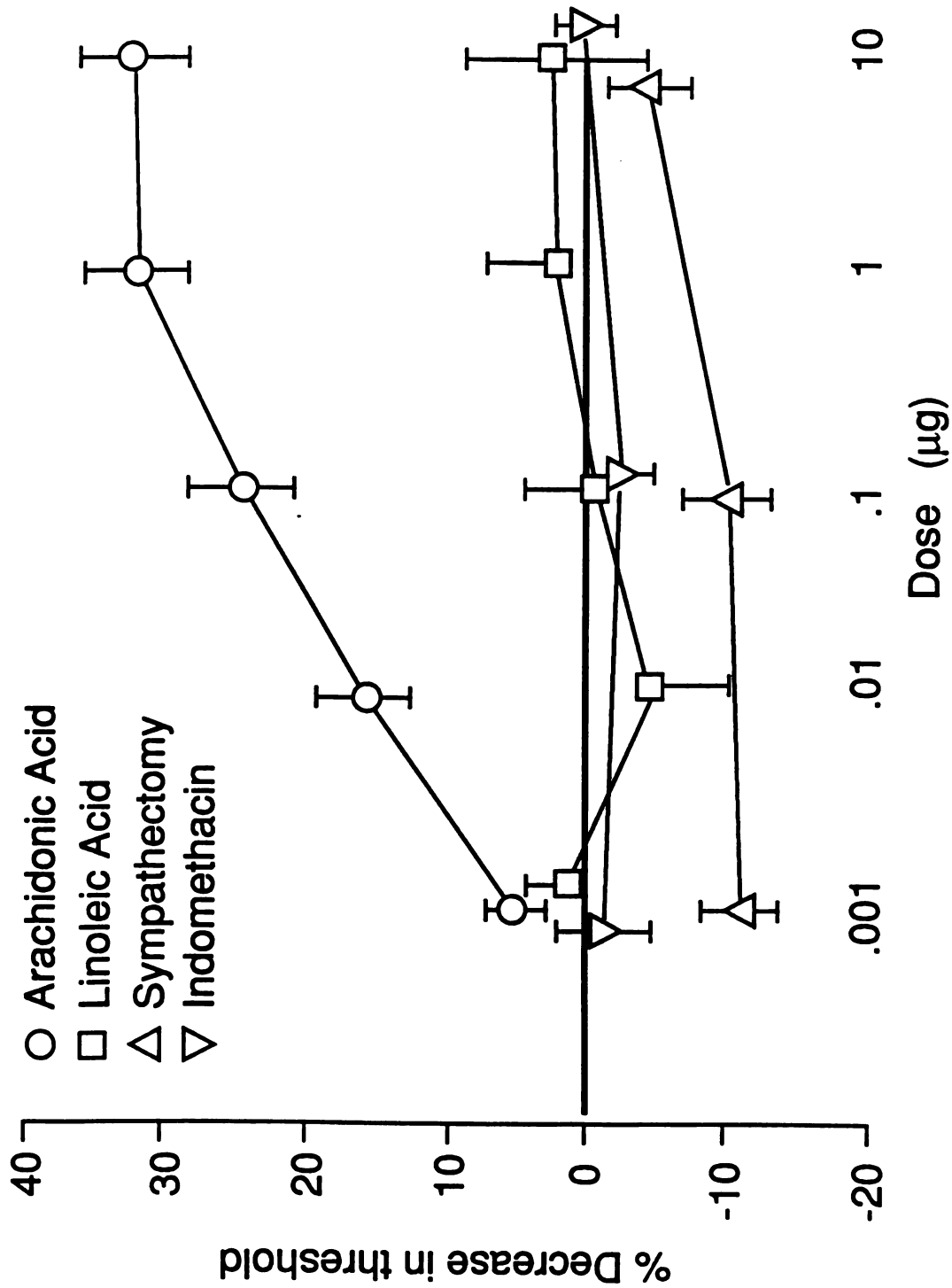

○ Arachidonic Acid
□ Linoleic Acid
△ Sympathectomy
▽ Indomethacin
% Decrease in threshold
40
30
20
10
0
-10
-20
.001
.01
.1
1
10
Dose (µg)

FIGURE 2

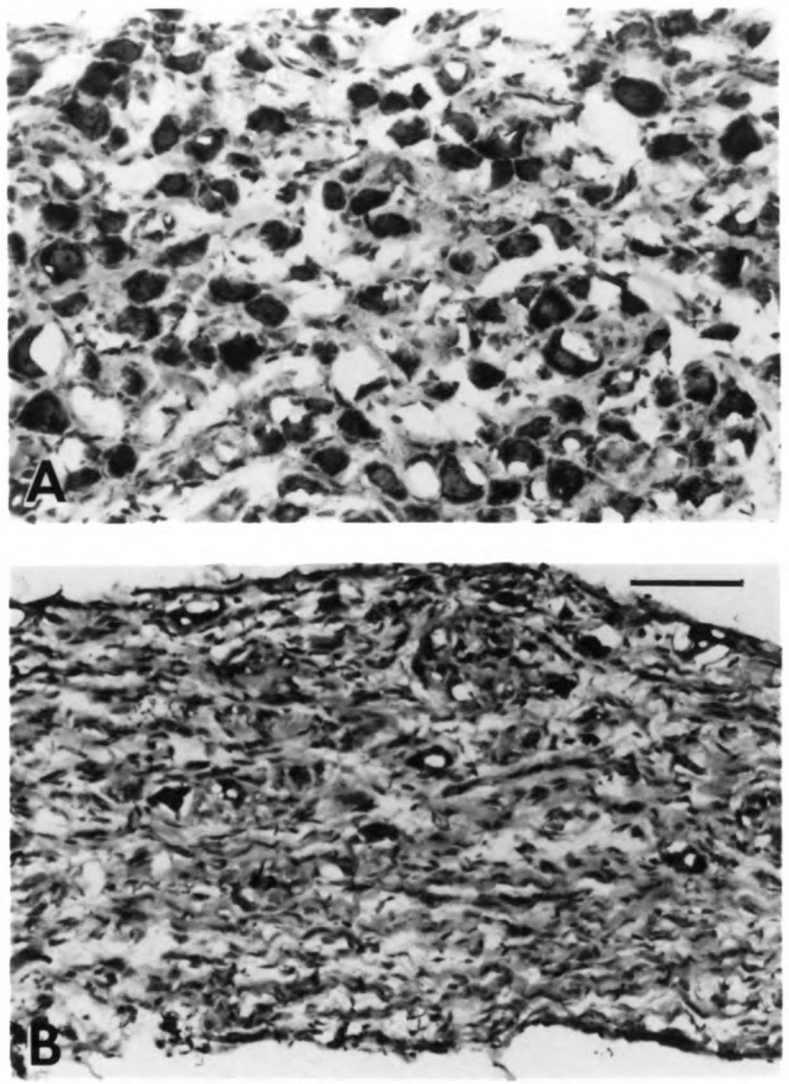


TABLE 1

TREATMENT	Prostaglandin E_2 Content (mean±SEM) (pg/ganglia	(pg/mg weight)
NORMAL	780±40 (n=4)	840±115 (n=4)
6-HYDROXYDOPAMINE	<62.5** (n=3)	<61.0** (n=3)

TREATMENT	6-keto-Prostaglandin F_{1a} Content (mean±SEM) (pg/ganglia)	(pg/mg weight)
NORMAL	490±20 (n=4)	480±20 (n=4)
6-HYDROXYDOPAMINE	80±17 (n=3)	300±46 (n=3)

*Prostaglandin content of ganglia prior to homogenization was, in all cases, below detection limits for both PGE_2 and 6-keto-PGF_{1a}.

FIGURE 3a

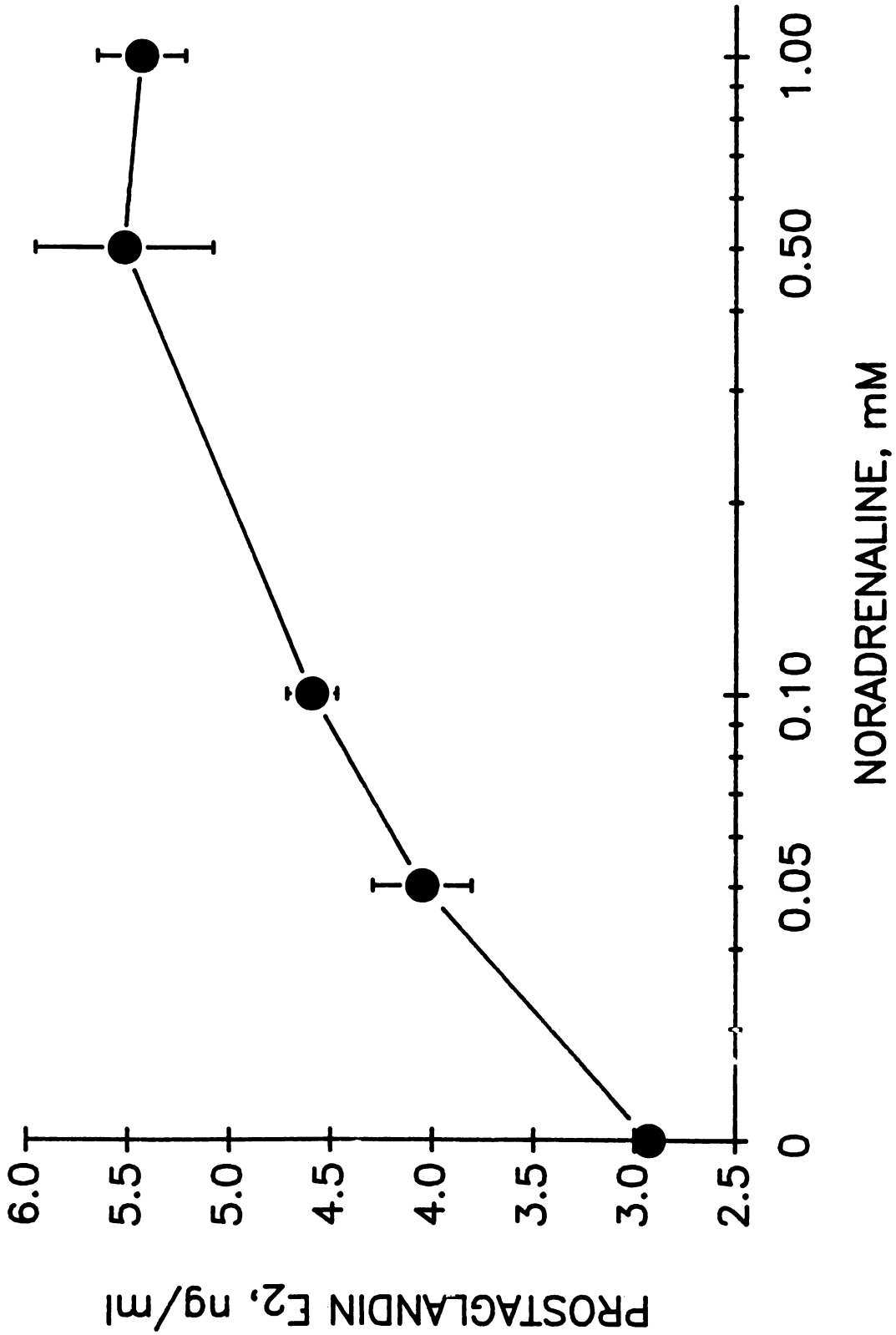

PROSTAGLANDIN E2, ng/ml
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
0
0.05
0.10
0.50
1.00
NORADRENALINE, mM

FIGURE 3b

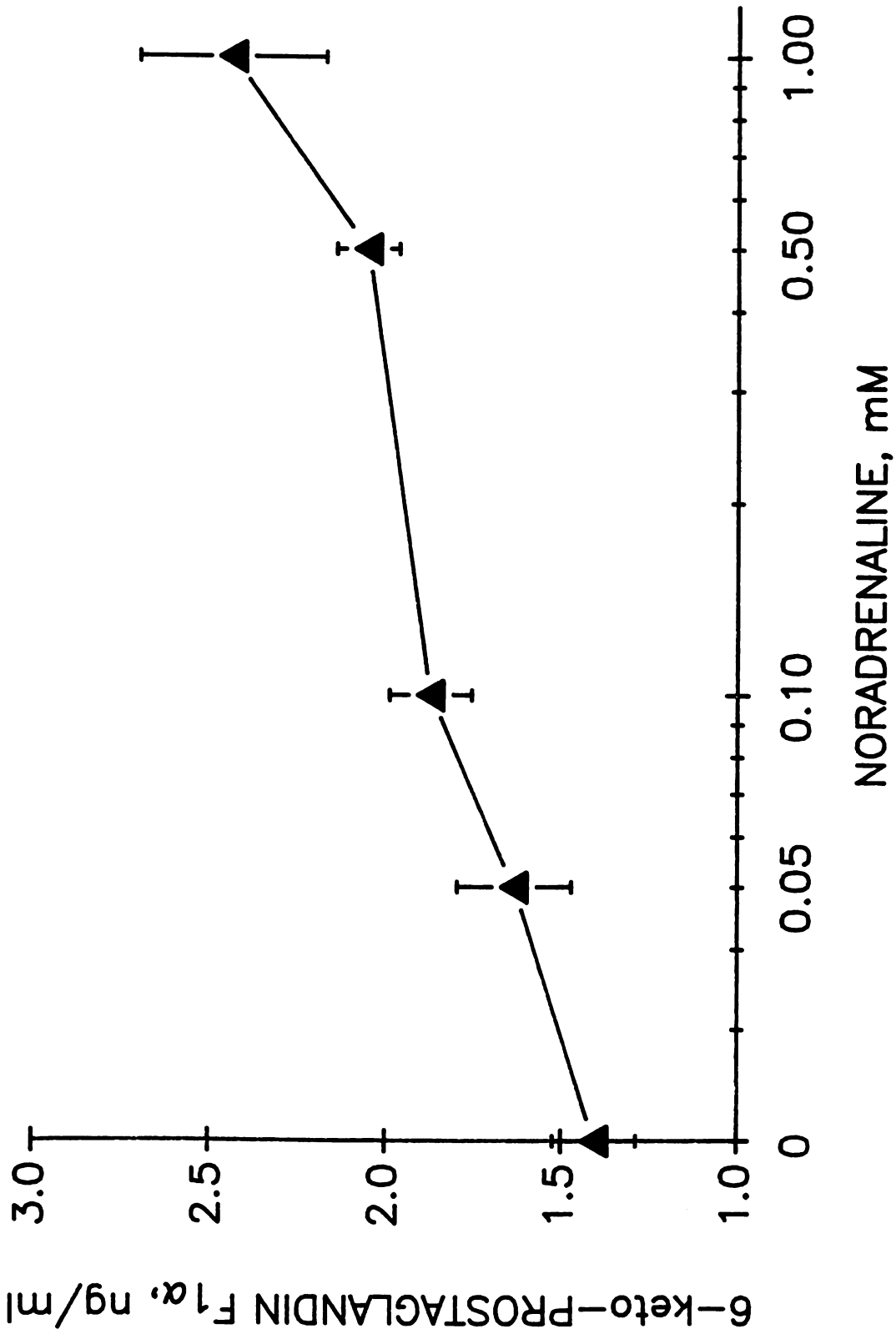

6-keto-PROSTAGLANDIN F1α, ng/ml
3.0
2.5
2.0
1.5
1.0
0
0.05
0.10
0.50
1.00
NORADRENALINE, mM

FIGURE 4a

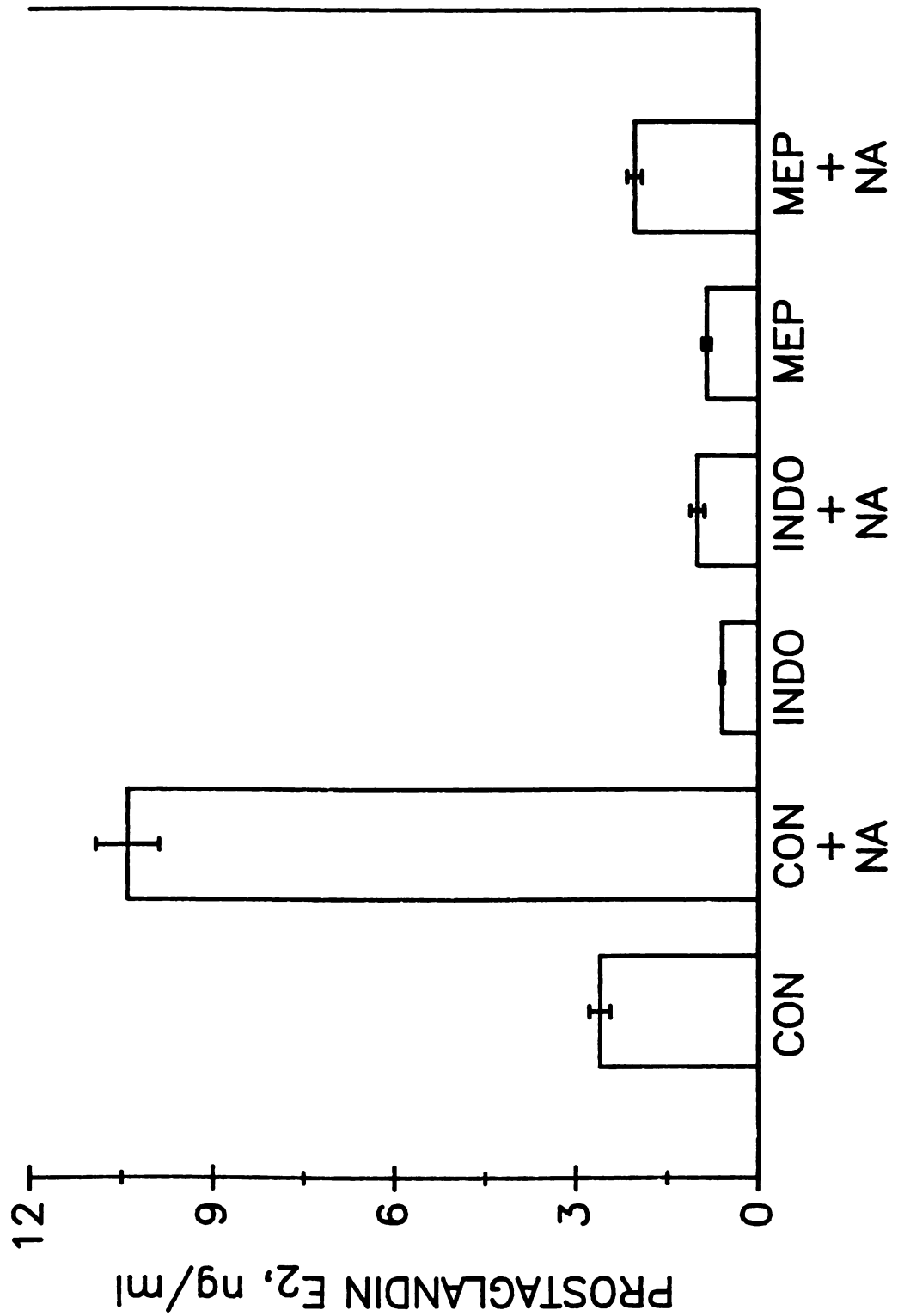

PROSTAGLANDIN E2, ng/ml
0
3
6
9
12
CON
CON + NA
INDO
INDO + NA
MEP
MEP + NA

FIGURE 4b

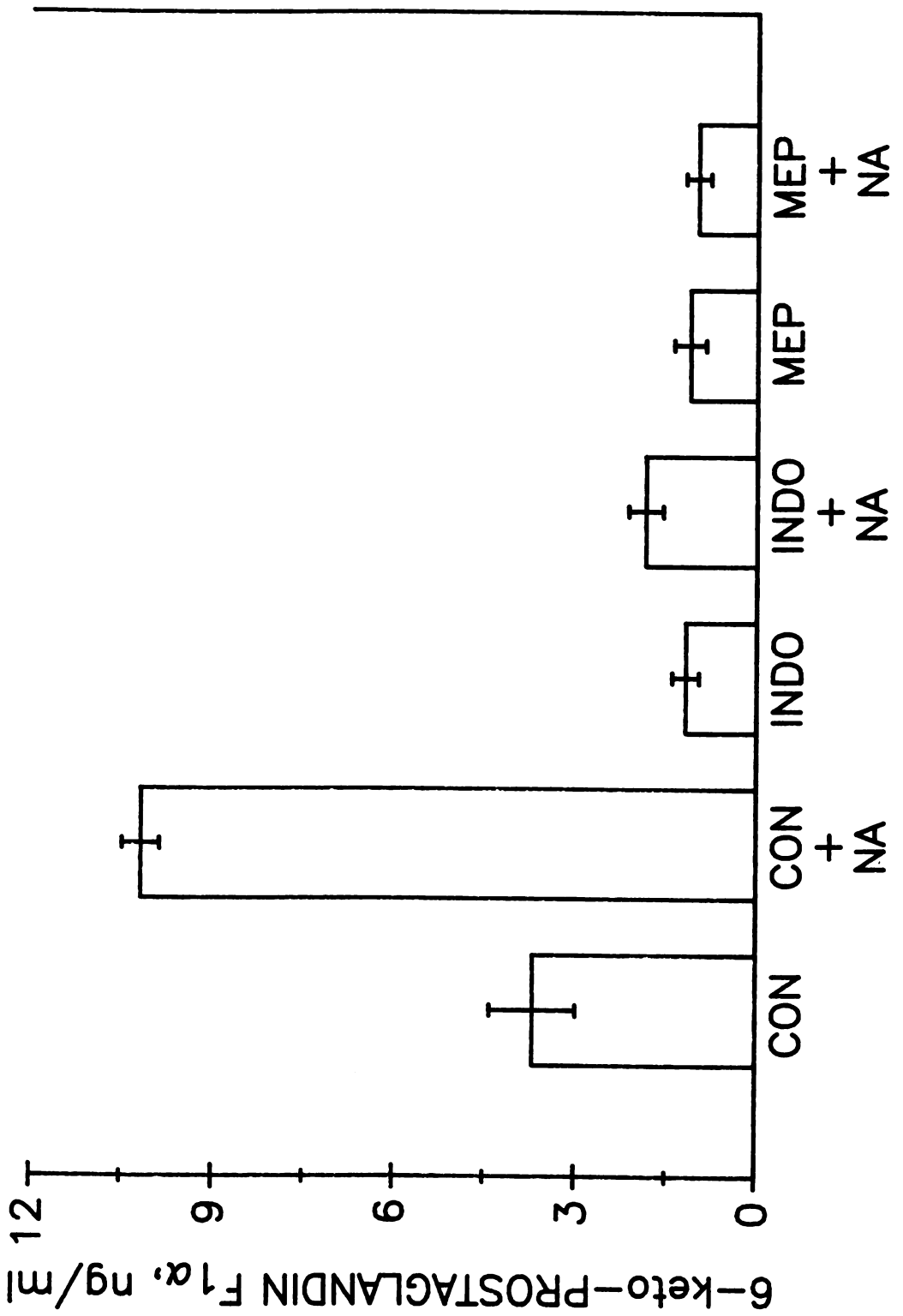

6-keto-PROSTAGLANDIN F1α, ng/ml
0
3
6
9
12
CON
CON + NA
INDO
INDO + NA
MEP
MEP + NA

FIGURE 5a

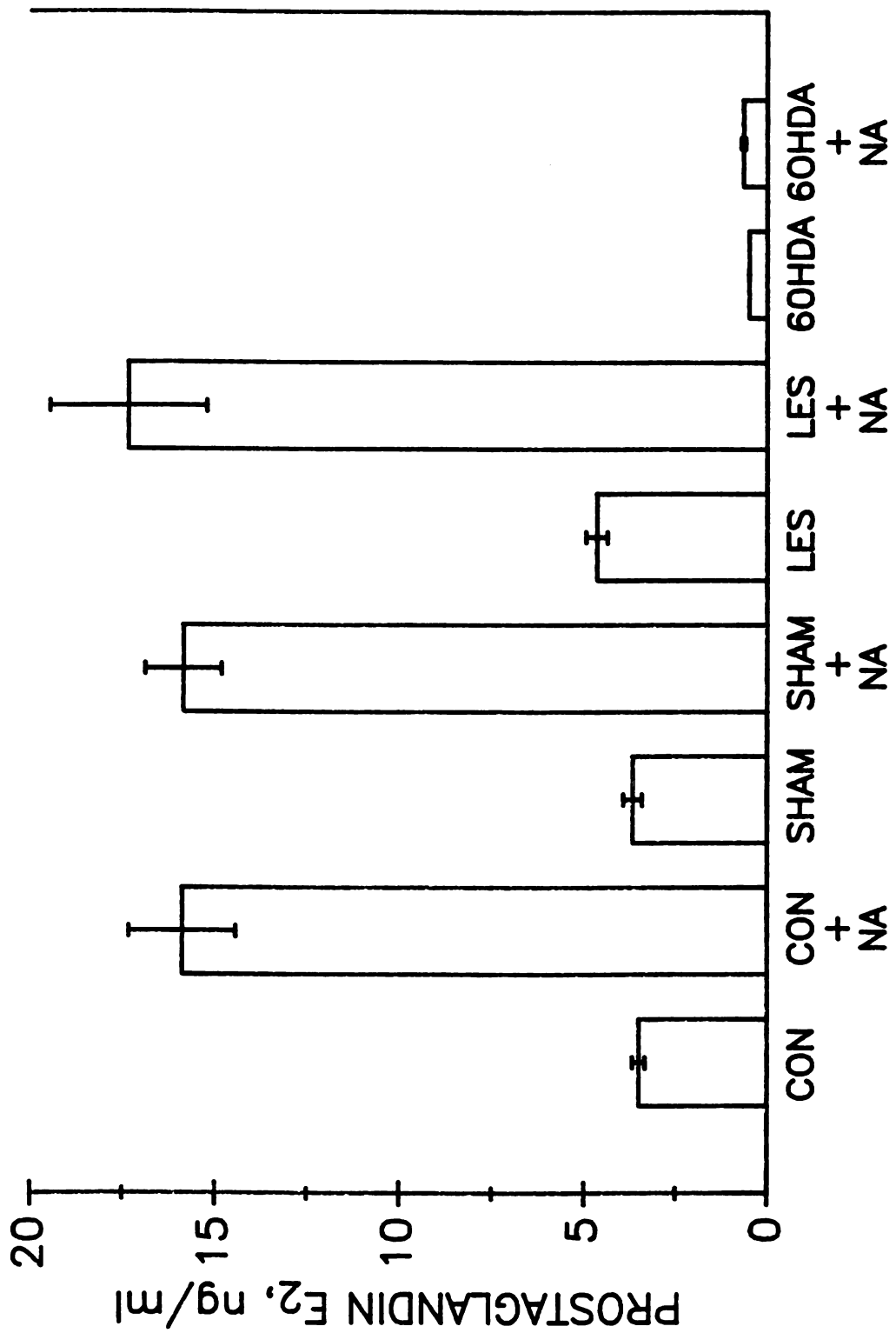

PROSTAGLANDIN E2, ng/ml
0
5
10
15
20
CON
CON + NA
SHAM
SHAM + NA
LES
LES + NA
6OHDA
6OHDA + NA

FIGURE 5b

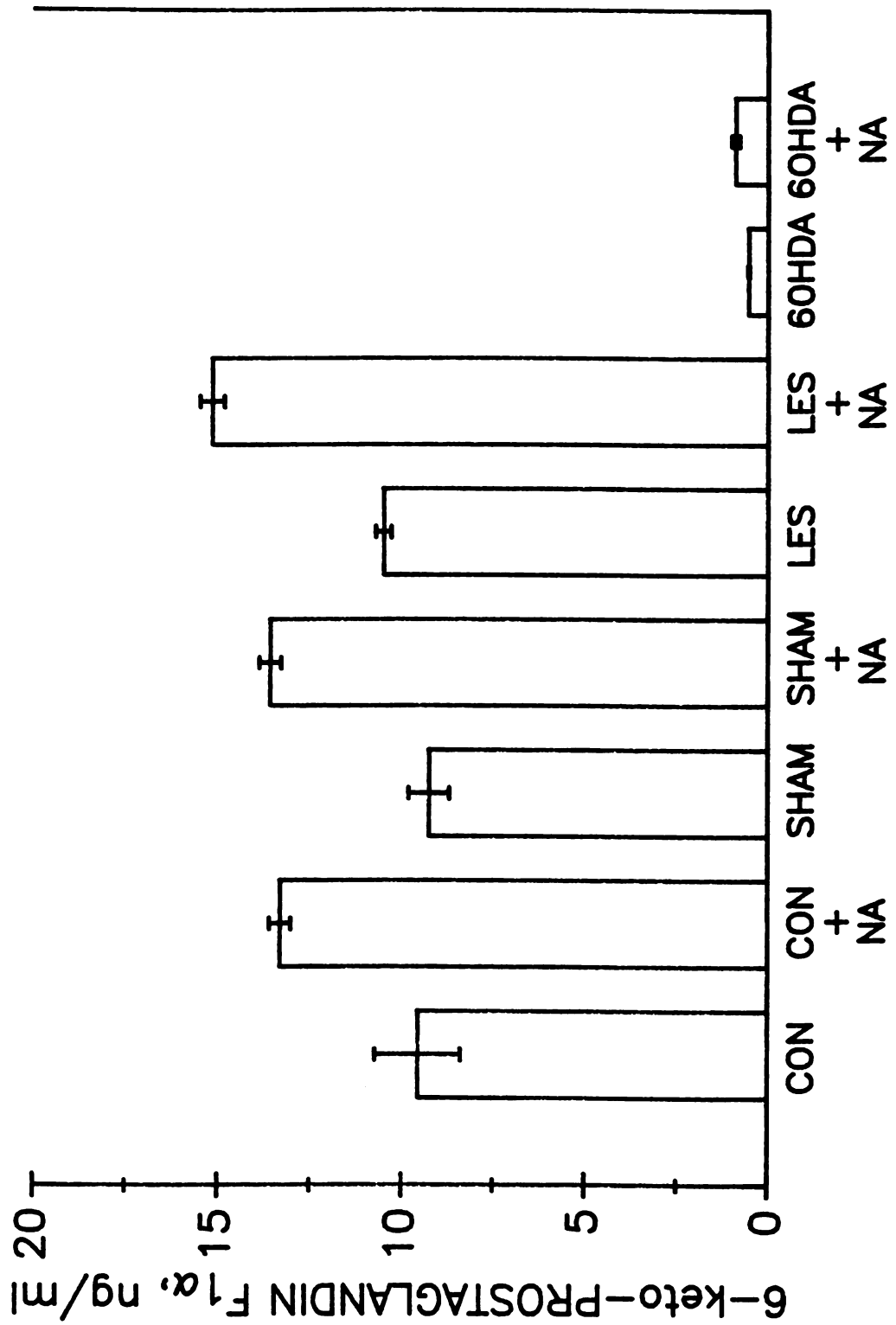
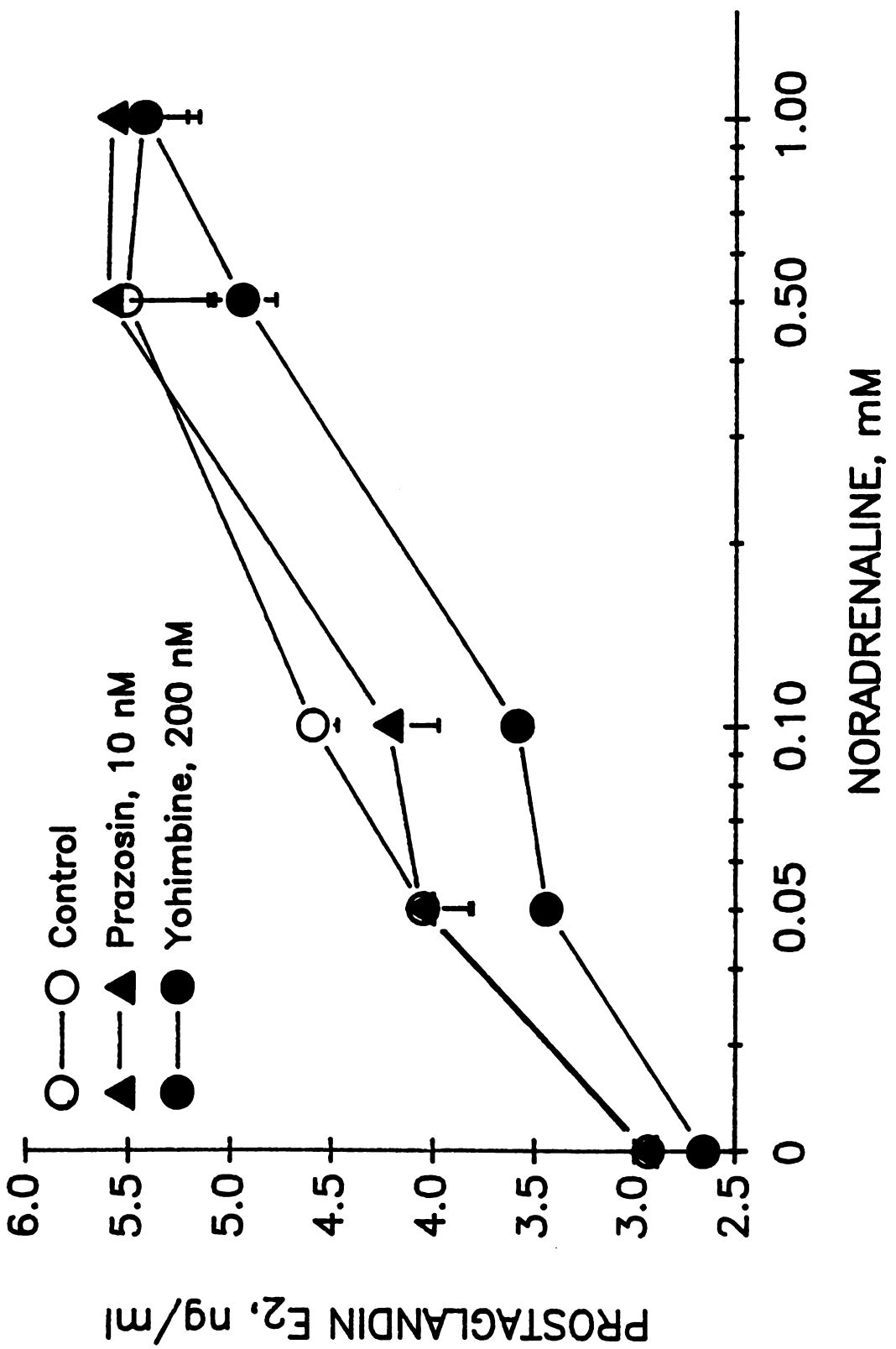

6-keto-PROSTAGLANDIN F1α, ng/ml
0
5
10
15
20
CON
CON + NA
SHAM
SHAM + NA
LES
LES + NA
6OHDA
6OHDA + NA

FIGURE 6


Control
Prazosin, 10 nM
Yohimbine, 200 nM
PROSTAGLANDIN E2, ng/ml
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
0
0.05
0.10
0.50
1.00
NORADRENALINE, mM

REFERENCES

Angeletti P.U. and Levi-Montalcini R. (1970) Sympathetic nerve cell destruction in newborn mammals by 6-OHDA. Proc. Natl. Acad. Sci. USA **65**, 114-121.

Ashe J.H. and Libet B. (1979) A noradrenergic s-IPSP in mammalian sympathetic ganglia elicited by a non-muscarinic action of preganglionic volleys. Soc. Neurosci. Abstr. **5**,735.

Ashe J.H. and Libet B. (1981) Modulation of slow postsynaptic potentials by dopamine, in rabbit sympathetic ganglia. Brain Res. **217**, 93-106.

Basran G.S., Paul W., Morley J., et. al. (1982) Evidence in man of synergistic interaction between putative mediators of acute inflammation and edema. Lancet **1**, 935-937.

Belluzzi O., Biondi S., Borasio P.G., et. al. (1982) Electrophysiological evidence for a PGE-mediated presynaptic control of acetylcholine output in the guinea-pig superior cervical ganglion. Brain Res. **236**, 383-391.

Berström S., Duner H., von Euler U.S., et. al. (1959) Observations of the effects of infusion of prostaglandin E in man. Acta. Physiol. Scand. **45**, 145-151.

Bunting S., Gryglewski R., Moncada S., et. al. (1976) Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. Prostaglandins **12**, 897-913.

Coceani R., Pace-Asciak C., Volta R. and Wolfe L.S. (1967) Effect of nerve stimulation on prostaglandin formation and release from the rat stomach. Am. J. Physiol. **213**, 1056-1064.

Coderre, T.J., Basbaum, A.I. and Levine, J.D. (1989) Neural control of vascular permeability: Interactions between primary afferents, mast cells, and sympathetic efferents. J. Neurophysiol. **62**, 48-58.

Davies B.N., Horton E.W. and Withrington P.G. (1968) The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. Br. J. Pharmacol. **32**, 127-135.

Davies P., Bailey P.J. and Goldenberg M.M. (1984) The role of arachidonic acid oxygenation products in pain and inflammation. Ann. Rev. Immunol. **2**, 335-357.

Davis H.A. and Horton E.W. (1972) Output of prostaglandins from the rabbit kidney, its increase on renal nerve stimulation and its inhibition by indomethacin. Br. J. Pharmacol. **46**, 658-675.

Dinarello C.A. and Wolff S.M. (1978) Pathogenesis of fever in man. New Engl. J. Med. **298**, 607-612.

Dun N.J. (1980) Inhibition of ACh release by PGE_1 in the rabbit superior cervical ganglion. Neuropharmacology **19**, 1137-1140.

Ferreira S.H. (1972) Prostaglandins, aspirin-like drugs and analgesia. Nature (New Biology) **240**, 200-203.

Ferreira S.H., Moncada S. and Vane J.R. (1973) Prostaglandins and the mechanism of analgesia produced by aspirin-like drugs. Brit. J. Pharmacol. **49**, 86-97.

Ferreira S.H., Nakamura M. and Castro M.S.A. (1978) The hyperalgesic effects of prostacyclin and prostaglandin E_2. Prostaglandins **16**, 31-37.

Ferreira S.H. and Vane J.R. (1967) Prostaglandins: Their disappearance from and release into the circulation. Nature **216**, 868-873.

Flower R.J. (1974) Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev. **26**, 33-67.

Frame M.H. and Hedqvist P. (1975) Evidence for prostaglandin mediated prejunctional control of renal vascular sympathetic tone. Br. J. Pharmacol. **54**, 189-196.

Gilmore N., Vane J.R. and Wyllie J.H. (1968) Prostaglandins released by spleen. Nature **218**, 1135-1140.

Goldberg M.R. and Robertson D. (1983) Yohimbine: A pharmacological probe for study of the alpha$_2$-adrenoreceptor. Pharmacol. Rev. **35**, 143-180.

Goldyne M.E., Winklemann R.K. and Ryan R.J. (1973) Prostaglandin activity in human cutaneous inflammation: Detection by radioimmunoassay. Prostaglandins **4**, 737-749.

Golub M.S., Berger M.E. and Powell M. (1985) Adrenergic stimulation of prostacyclin production in the rat artery. I. Response to antagonists. Prostaglandins Leukotrienes Med. **20**, 299-311.

Greenberg R. (1978) The neuronal origin of prostaglandin released from the rabbit portal vein in response to electrical stimulation. Br. J. Pharmacol. **63**, 79-85.

Hedqvist P. (1970) Studies on the effect of prostaglandins E_1 and E_2 on the sympathetic neuromuscular transmission in some animal tissues. Acta Physiol. Scand. **79, suppl. 345**, 1-40.

Hedqvist P. (1974) Prostaglandin action on noradrenaline release and mechanical responses in the stimulated guinea-pig vas deferens. Acta Physiol. Scand. **90**, 86-93.

Hedqvist P. (1977) Basic mechanism of prostaglandin action on autonomic neurotransmission. Ann. Rev. Pharmacol. Toxicol. **17**, 259-279.

Hedqvist P., Stjarne L. and Wennmalm A. (1971) Facilitation of sympathetic neurotransmission in the cat spleen after inhibition of prostaglandin synthesis. Acta Physiol. Scand. **83**, 430-432.

Jeremy J.Y., Mikhailidis D.P. and Dandona P. (1985) Adrenergic modulation of vascular prostacyclin (PGI_2) secretion. Eur. J. Pharmacol. **114**, 33-40.

Junstad M. and Wennmalm A. (1973) On the release of prostaglandin E_2 from the rabbit heart following infusion of noradrenaline. Acta Physiol. Scand. **87**, 573-574.

Kuhn D.C. and Willis A.L. (1973) Prostaglandin E_2, inflammation and pain threshold in rat paws. Brit. J. Pharmacol. **49**, 183P.

Levine J.D., Taiwo Y.O., Collins S., et. al. (1986) Noradrenaline hyperalgesia is mediated through interaction with sympathetic postganglionic neurone terminals rather than activation of primary afferent nociceptors. Nature **323**, 158-160.

Loh L. and Nathan P.W. (1978) Painful peripheral states and sympathetic blocks. J. Neurol. Neurosurg. Psychiat. **41**, 664-671.

Malik K.U. (1978) Prostaglandins-modulation of adrenergic nervous system. Fed. Proc. **39**, 203-207.

Milton A.S. (1978) The role of prostaglandins in pyrexia. Biochem. Soc. Trans. **6**, 727-731.

Mo M., Ammari R. and Dun N.J. (1985) Prostaglandin E_1 inhibits calcium-dependent potentials in mammalian sympathetic neurons. Brain Res. **334**, 325-329.

Mochida S., Kobayashi H. and Libet B. (1987) Stimulation of adenylate cyclase in relation to dopamine-induced long-term enhancement (LTE) of muscarinic depolarization in the rabbit superior cervical ganglion. J. Neurosci. **7**, 311-318.

Moncada S. and Higgs E.A. (1988) Metabolism of arachidonic acid. Annals NY Acad. Sci. **522**, 454-463.

Moncada S. and Vane J.R. (1979) Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin. Pharmocol. Rev. **30**, 293-331.

Needleman P., Douglas J.R., Jr., Jakschik B., et. al. (1974) Release of renal prostaglandin by catecholamines: relationship to renal endocrine function. J. Pharmacol. Exp. Ther. **188**, 453-460.

O'Brien J.R. (1968) Effects of salicylates on human platelets. Lancet **1**, 779-781.

Petkov V. and Radomirov R. (1980) On the origin of prostaglandin and its role in the sympathetic nerve transmission in vas deferens. Gen. Pharmacol. **11**, 275-282.

Pipili E. and Poyser N.L. (1987) Differential effects of prazosin and rauwolsin on the release of prostaglandins I_2 and E_2 from the perfused mesenteric arterial bed of the rabbit following nerve stimulation. Prostaglandins **23**, 300-390.

Quik M., Weldon P. and Collier B. (1987) Target organ destruction enhances recovery of choline acetyltransferase activity in adult rat sympathetic ganglia after denervation. Expt. Neurol. **95**, 178-193.

Rump L.C. (1987) Modulation of renal noradrenaline release by prejunctional receptor systems *in vitro*. Clin. Exptl. Pharmacol. Physiol. **14**, 423-428.

Somova L. (1973) Inhibition of prostaglandin synthesis in the kidneys by aspirin-like drugs. Advances in the Biosciences, Vol. **9** (Suppl.) (Bergstrom S. and Bernhard S., eds), Pergamon Press, Vienna, p. 53.

Starke K. (1977) Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol., Biochem., Pharmacol. **77**, 1-124.

Starke K. (1981) α-Adrenoceptor subclassification, Rev. Physiol. Biochem. Pharmacol. **88**, 199-236.

Stjarne L. (1972) Prostaglandin E restricting noradrenaline secretion--neural in origin? Acta Physiol. Scand. **86**, 574-576.

Taiwo Y.O. and Levine J.D. (1988) Characterization of the arachidonic acid metabolites mediating bradykinin and noradrenaline hyperalgesia. Brain Res. **458**, 402-406.

Taniguchi T., Nishikawa H., Yokotani K., et. al. (1988) The bindings of ^{3}H-prazosin and ^{3}H-yohimbine to alpha adrenoceptors in the guinea-pig stomach. Life Sciences **42**, 2341-2347.

Trachte G.J. (1987) Adrenergic receptors mediating prostaglandin production in the rabbit vas deferens. Prostaglandins **33**, 25-35.

Trevisani A., Biondi C., Belluzzi O., et. al. (1982) Evidence for increased release of prostaglandins of E-type in response to orthodromic stimulation of the guinea-pig superior cervical ganglion. Brain Res. **236**, 375-381.

Vargaftig B. and Zirinis P. (1973) Platelet aggregation induced by arachidonic acid is accompanied by release of potential inflammatory mediators distinct from PGE_2 and $PGF_{2\alpha}$. Nature **244**, 114-116.

von Euler U.S. (1934) Zur Kenntnis der pharmakologischen Wirkungen von Nativsekreten und extrakten mannlischer accessorischer Geschlechtsdrusen. Naunyn-Schmiedeberg's Arch. Pharmacol. **175**, 78-84.

von Euler U.S. (1935) Uber die specifische Blutdrucksenkende Substantz des menschlichen Prostata- and Samenblasensekretes. Klin. Wochenschr. **14**, 1182-1183.

von Euler U.S. (1936) On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). J. Physiol. (Lond.) **88**, 213-234.

Webb J.G., Saelens D.A. and Halushka P.V. (1978) Biosynthesis of prostaglandin E by rat superior cervical ganglia. J. Neurochem. **32**, 13-19.

Wedmore C.V. and Williams T.J. (1981) Control of vascular permeability by polymorphonuclear leukocytes in inflammation. Nature **289**, 646-650.

Wennmalm A. (1971) Studies on mechanisms controlling the secretion of neurotransmitters in the rabbit heart. Acta Physiol. Scand. **82, Suppl. 365**, 1-36.

Wennmalm M., Fitzgerald G.A. and Wennmalm A. (1987) Prostacyclin as neuromodulator in the sympathetically stimulated rabbit heart. Prostaglandins **33**, 675-691.

Wiesenfeld-Hallin Z. and Hallin R.G. (1984) The influence of the sympathetic nervous system on mechanoreception and nociception. A Review. Human Neurobiol. **3**, 41-46.

Williams T.J. and Peck M.J. (1973) Role of prostaglandin-mediated vasodilation in inflammation. Nature **270**, 530-532.

Willis A.L., Davison P., Ramwell P.W., et. al. (1972) Release and actions of prostaglandins in inflammation and fever: Inhibition by anti-inflammatory and anti-pyretic drugs. In Prostaglandins in Cellular Biology (Ramwell P.W. and Pharriss B.B., eds), Plenum Press, New York., pp. 227-259.