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μ- and δ-opioid receptor-mediated contractile effects on rat aortic vascular smooth muscle

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Abstract

The actions of opioid receptor agonists and antagonists were studied in isolated rat aortic strips. Morphine (10^{-7}–10^{-6} M) had no contractile effect on resting strips but when added during the relaxation of the contractions induced by 10^{-9} M noradrenaline, it induced a contractile response which was blocked by naloxone. The selective μ-opioid receptor agonist, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin (DAMGO, 10^{-7}–10^{-6} M), induced an increase in basal tension which remained after removal of endothelium or in Ca^{2+}-free solution, but was inhibited by β-flunaltrexamine. β-Flunaltrexamine also inhibited the contractile response induced by DAMGO added during the relaxation of the contractions induced by noradrenaline. The δ-opioid receptor agonist, [D-Pen^2,D-Pen^5]enkephalin, had no effect on resting tension but potentiated the contractions induced by noradrenaline; these effects were abolished by naltrindol. The selective κ-opioid receptor agonist, bremazocine, had no effect on resting tension and did not modify the amplitude of the contractions induced by noradrenaline. These results suggest that, at low concentrations, agonists of μ- and δ-opioid receptors may act as modulators of noradrenaline-induced responses, whereas at higher concentrations, μ-opioid receptor stimulation may have a direct contractile effect in isolated rat aorta.

Keywords: Morphine; Opioid receptor; Aorta, rat; Contractile force

1. Introduction

The endogenous opioid system participates in the regulation of vascular smooth muscle tone, regional blood flow and blood pressure in normal and hypertensive states (Martin, 1976; Holaday, 1983; Johnson et al., 1985; Feuerstein and Siren, 1987; Reid et al., 1984; Lang et al., 1982; Olson et al., 1989). In spontaneously hypertensive rats (SHR) there is an increased vascular responsiveness to opioid peptides, and opioid receptor antagonists produce a more pronounced hypotensive response than they do in normotensive Wistar-Kyoto rats (Yukimura et al., 1981; Lang et al., 1982; Levin et al., 1986; Feuerstein and Siren, 1987). These findings further indicate an involvement of the endogenous opioid system in the pathogenesis of essential hypertension (Johnson et al., 1985; Levin et al., 1986; Feuerstein and Siren, 1987; Olson et al., 1989). Moreover, the competitive opioid receptor antagonist, naloxone, has been used as a probe to examine the possible role of opioid peptides in the development of hypotension and myocardial depression in various forms of shock (haemorraghic and endotoxic), the fall in blood pressure during sleep and the antihypertensive effects of clonidine or α-methyldopa (Holaday and Faden, 1978; Farsang et al., 1982; Lang et al., 1982; Rubin, 1984). However, some confusion still exists with respect to the cardiovascular action of opioids, that is, opposite effects can be observed on blood pressure and heart rate depending on the opiod used, dosage, animal species, route of administration or the type of anesthesia used (Lang et al., 1982; Holaday, 1983; Reid et al., 1984; Feuerstein and Siren, 1987; Olson et al., 1989).

Although most attention has been focused on their central site of action, a peripheral site(s) of action of opioids cannot be ruled out. In this regard, the direct effects of opioids on vascular smooth muscle tone have

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been poorly investigated. Moreover, in isolated vessels the effect of opioids on vascular tone varies according to species, and even in the same species, between different vascular beds (Lee and Berkowitz, 1976; Altura et al., 1978; Harder and Madden, 1984; El-Sharkawy et al., 1991). Additionally, little is known regarding the opiod receptor subtypes (μ-, δ- and κ-opioid binding sites) involved in these actions (Haynes, 1988). Isolated rat aorta has a sparse or absent innervation (Webb et al., 1983) and therefore permits the direct effects of opioid receptor agonists and antagonists on vascular smooth muscle to be tested, i.e. with minimum interference with prejunctional nerves. In an attempt to better define and characterize the vascular effects of opioids, we have now examined the effects of several opioid receptor agonists and antagonists on contractile responses in isolated rat aorta. A preliminary report of these results has been presented (Parra et al., 1993).

2. Material and methods

2.1. Tissue preparation

Male Wistar rats (200–250 g) from Interfauna (Barcelona, Spain) were used. After decapitation, the descending thoracic aorta was rapidly dissected and placed in a physiological saline solution (PSS) of the following composition (mM): NaCl 118, KCl 4.75, $NaHCO_3$ 23.8, $MgSO_4$ 1.2, $CaCl_2$ 2, KH_2PO_4 1.2 and glucose 11, previously bubbled with a 95% O_2-5% CO_2 gas mixture. After excess surrounding tissue was removed, the aortae were cut into two spiral strips (2–3 mm wide, 20 mm in length) by the method of Furchgott and Bhadrakom (1953). Care was taken to avoid endothelial damage except in some aortae in which the endothelium was removed by gently rubbing the internal surface of the vessels with a glass rod. In these latter experiments, the absence of a functional endothelium was confirmed at the beginning of the experiment by the inability of the preparation, precontracted with 10^{-6} M noradrenaline, to relax in response to 10^{-6} M acetylcholine.

2.2. Mechanical force recording

Aortic helical strips were mounted vertically under 2 g of tension in 10-ml organ baths filled with PSS, maintained at 37° C, and bubbled with a 95% O_2-5% CO_2 gas mixture. Isometric tension was recorded on a Grass polygraph (Model 7D) through force-displacement transducers (Grass FT07) as previously described (Pérez-Vizcaíno et al., 1991,1993). The preparations were allowed to equilibrate for 60 min prior to initiation of experimental procedures, and during this period the incubation media were changed every 15–30 min.

Morphine as well as selective μ- ([D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin, DAMGO), δ- ([D-Pen^2,D-Pen^5]enkephalin, DPDPE) and κ- (bremazocine) opioid receptor agonists were tested in different experimental protocols. We also explored the effects of naloxone as well as selective μ- (β-flunaltrexamine) and δ- (naltrindol) opioid receptor antagonists.

After equilibration of the preparations the following experiments were performed: (a) the effects of four agonists (morphine, DAMGO, DPDPE and bremazocine) in a range of concentrations between 10^{-7} M and 10^{-6} M were studied on basal tension. Agonists were added to the bath cumulatively in the absence or presence of their respective selective antagonists. The effects of 10^{-6} M DAMGO on basal tension were also studied in resting aortic strips in which the endothelium was mechanically removed and in muscles incubated for 10 min in Ca^{2+}-free 0.1 mM EDTA PSS. (b) In another set of experiments, aortic strips were exposed to 10^{-9} M noradrenaline for 5 min and then washed for the next 30 min. Control contractile responses were obtained at the beginning of the experiment until two successive responses were almost identical in height. This was followed by exposure to an opioid receptor agonist for 10 min before addition of noradrenaline to the bathing media. When opioid receptor antagonists were tested they were added 15 min before the addition of noradrenaline. Only one agonist or antagonist was tested in each muscle. At 10^{-9} M noradrenaline induced a fast contractile response which reached a peak in 3–5 min and then slowly relaxed. (c) In some experiments the agonists were added when the relaxation phase of noradrenaline-induced responses reached about 50% of the peak contraction. In some muscles, the effects of morphine and DAMGO added when the contractile response induced by 10^{-9} M noradrenaline reached its maximal tension were studied. (d) In some strips, a contractile response to 20 mM KCl was elicited. When the contraction was stable, the effects of a single concentration of morphine (10^{-6} M) were studied.

2.3. Drugs

The following drugs were used: noradrenaline bitartrate, acetylcholine chloride, bremazocine and naloxone hydrochloride (Sigma Chemical Co., London, UK), morphine hydrochloride (Alcaliber, Spain), DAMGO and DPDPE (Bachem, Feinchemikalien, Bubendorf, Switzerland), β-flunaltrexamine hydrochloride and naltrindol (Research Biochemicals, MA, USA). All drugs were dissolved in distilled deionized water as a 10^{-2} M stock solution, except β-flunaltrexamine, which was initially dissolved in methanol. Further dilutions were

made in PSS. Ascorbic acid (10^{-5} M) was added to each stock solution of noradrenaline to prevent degradation.

2.4. Statistical analysis

The data are expressed as means ± S.E.M. Statistical significance of differences was assessed by one-way analysis of variance followed by Scheffe's *t*-test. A value of $P < 0.05$ was taken to indicate significance.

3. Results

3.1. Effects of morphine

Morphine, when added to resting aortic strips at concentrations between 10^{-7} M and 10^{-6} M, had no effect on resting tension. Moreover, morphine 10^{-7}–10^{-6} M, added when the tonic contractile response induced by a maximally effective concentration of noradrenaline (10^{-6} M) reached steady state, had no measurable effects on the tension developed (920 ± 29 mg vs. 910 ± 30 mg; $n = 8$; $P > 0.05$). Pretreatment with 10^{-6} M morphine had no effect on the phasic contractile responses induced by 10^{-9} M noradrenaline (508 ± 30 mg vs. 562 ± 30 mg; $n = 7$; $P > 0.05$).

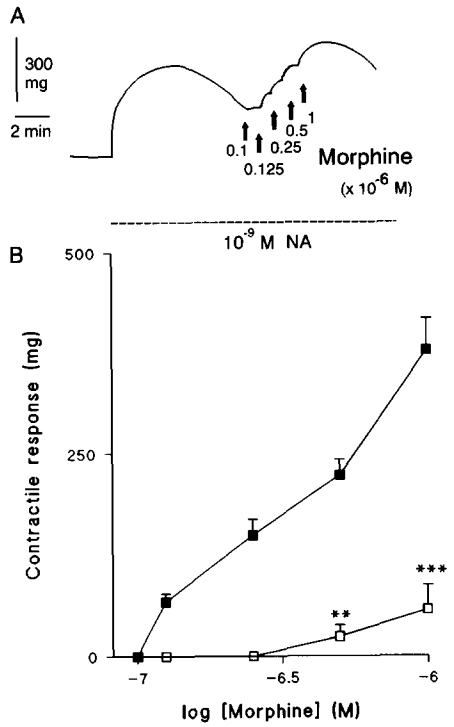

Fig. 1. Panel A shows an original recording of the effects of morphine on the phasic contractile responses induced by 10^{-9} M noradrenaline in rat aortic strips. Morphine, 10^{-7}–10^{-6} M, was added cumulatively when the contractile response to noradrenaline decayed by about 50% as indicated by the arrows. Panel B shows the averaged contractile responses induced by morphine in the absence (■) and in the presence (□) of 10^{-6} M naloxone. Abscissa: log morphine concentration (M). Ordinate: contractile force developed over the previous tension (mg). Each point represents the mean ± S.E.M. of 6–8 contractions. $^{**}P < 0.01$; $^{***}P < 0.001$.

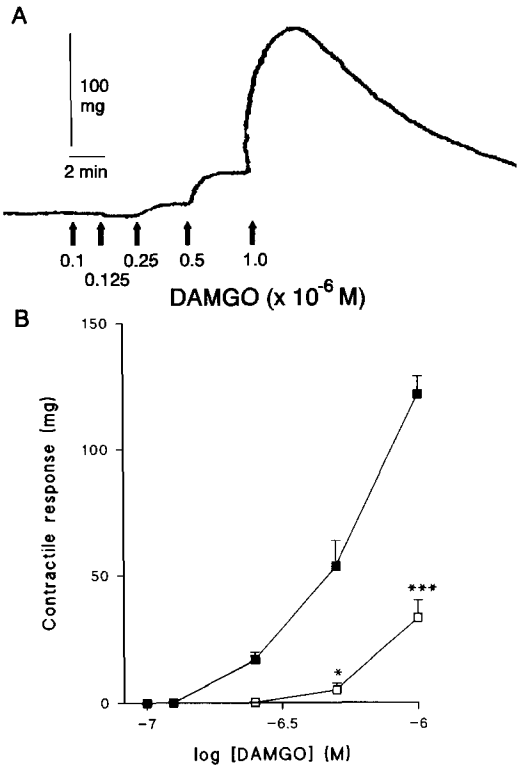

Fig. 2. Panel A shows an original recording of the effects of DAMGO, 10^{-7}–10^{-6} M, on resting tension when added cumulatively to rat aortic strips as indicated by the arrows. Panel B shows the contractile responses induced by DAMGO in the absence (■) and in the presence (□) of 10^{-6} M β-flunaltrexamine. Abscissa: log DAMGO concentration (M). Ordinate: contractile force developed (mg). Each point represents the mean ± S.E.M. of 6–8 contractions. $^{*}P < 0.05$; $^{***}P < 0.001$.

The phasic contractile responses to 10^{-9} M noradrenaline reached a peak in 3–5 min and were followed by a slow decay of tension. Thus, in another group of experiments, morphine (10^{-7}–10^{-6} M) was added to the bath when the relaxation phase of noradrenaline-induced contraction reached about 50% of the peak contraction. Under these conditions (Fig. 1A), morphine induced a concentration-dependent contractile response which reached values above the peak response to noradrenaline (668 ± 81 mg vs. 581 ± 80 mg; $n = 7$; $P > 0.05$). Fig. 1B also shows that the opioid receptor antagonist, naloxone (10^{-6} M), completely suppressed the contractile responses induced by 10^{-7} M and 5×10^{-6} M morphine, while those induced by higher concentrations of morphine were partially blocked by naloxone.

Addition of 20 mM KCl to aortic strips induced a stable contraction (480 ± 50 mg, $n = 4$). Afterwards,

addition of 10^{-6} M morphine induced a small contraction averaging $11.2 \pm 2.4\%$ over the previous control value ($P < 0.05$).

3.2. μ-Opioid receptor-selective agonists

In contrast to morphine, DAMGO (10^{-7}–10^{-6} M) induced a concentration-dependent contraction of resting strips (121 ± 7 mg at 10^{-6} M DAMGO, which represents a $14 \pm 2\%$ increase of the contraction induced by a maximally effective concentration of noradrenaline, e.g. 10^{-6} M). As shown in Fig. 2A, the increase in basal tension was fast but transient, reaching a peak in 2–4 min followed by a slow decay of tension. The increase in basal tension induced by DAMGO remained unaltered in strips incubated in Ca^{2+}-free PSS (Fig. 3). In endothelium-denuded arteries, 10^{-6} M DAMGO was still able to induce a contractile response of similar magnitude (not shown). Moreover, β-flunaltrexamine, a selective μ-opiod receptor antagonist, completely suppressed the contractile responses induced by 10^{-7} M and 5×10^{-6} M DAMGO, whereas it only partially suppressed the contractions induced by higher concentrations of DAMGO (Fig. 2B). When added during relaxation of the phasic contractile responses induced by 10^{-9} M noradrenaline, DAMGO (10^{-7}–10^{-6} M) also induced a concentration-dependent contractile response which was also inhibited by ß-flunaltrexamine (Fig. 4).

In another group of six experiments, DAMGO (10^{-7}–10^{-6} M) was added when the contractile response induced by 10^{-9} M noradrenaline reached its maximum amplitude (429 ± 55 mg; $n = 6$). Under these conditions DAMGO, 10^{-7}–10^{-6} M, produced a concentration-dependent increase in the tension developed, which reached significant values at 5×10^{-7} M (592 ± 61 mg; $P < 0.01$) and 10^{-6} M (747 ± 72 mg; $n = 6$; $P < 0.001$).

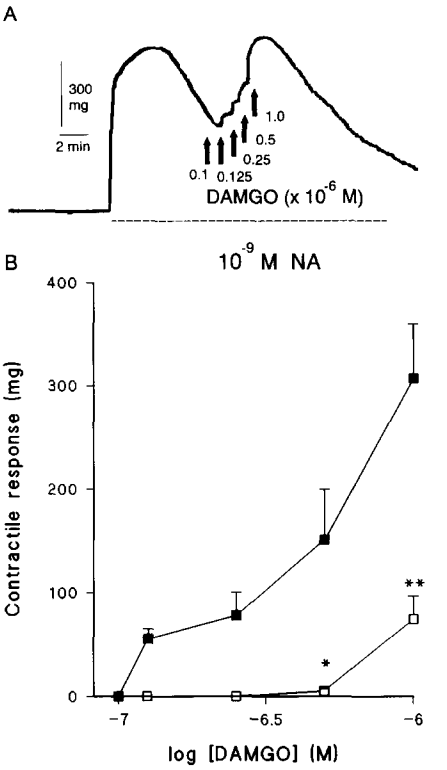

Fig. 4. Panel A shows an original recording of the effects of DAMGO when added cumulatively to rat aortic strips. Strips were precontracted with 10^{-9} M noradrenaline and DAMGO, 10^{-7}–10^{-6} M, was added cumulatively when the contractile response to noradrenaline had decayed by about 50% as indicated by the arrows. Panel B shows the averaged contractile responses induced by DAMGO in the absence (■) and in the presence (□) of 10^{-6} M β-flunaltrexamine. Abscissa: log DAMGO concentration (M). Ordinate: contractile force developed over the previous tension (mg). Each point represents the mean ± S.E.M. of 6–8 contractions. $^{*}P < 0.05$; $^{**}P < 0.01$.

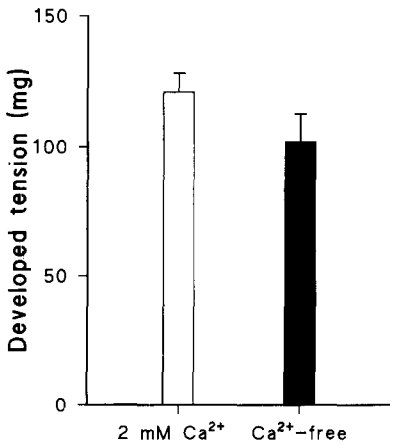

Fig. 3. Lack of effect of extracellular Ca^{2+} concentration on the contractile responses induced by 10^{-6} M DAMGO in resting rat aortic strips. Contractions induced by DAMGO were obtained in intact strips incubated in PSS (2 mM Ca^{2+}) or in Ca^{2+}-free PSS containing 0.1 mM EDTA. Each column is the mean ± S.E.M. of 5–7 experiments. Ordinate: tension developed (mg).

3.3. δ- and κ-selective opioid receptor agonists

The δ-opioid receptor agonist, DPDPE, at concentrations up to 10^{-6} M had no effect on resting tension and did not produce a contractile response when added at the relaxing phase of noradrenaline-induced contraction. However, as shown in Fig. 5, preincubation with 10^{-6} M DPDPE potentiated the amplitude of the phasic contractile response induced by 10^{-9} M noradrenaline. This effect was completely abolished in aortic strips pretreated with the selective δ-opioid receptor antagonist naltrindol (10^{-6} M). The selective κ-opioid receptor agonist bremazocine at concentrations up to 10^{-6} M had no effect on resting tension and did not modify the amplitude of the contractions

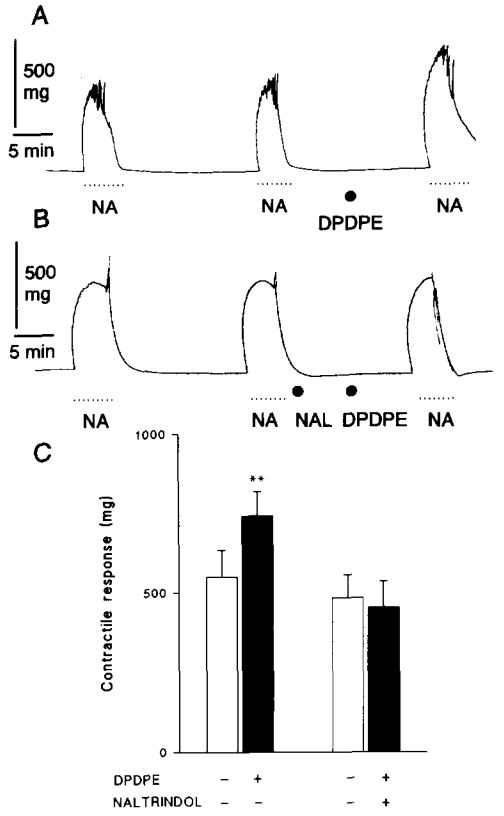

Fig. 5. Effect of 10^{-6} M DPDPE, a δ-selective opioid receptor agonist, on the contractions induced by 10^{-9} M noradrenaline (NA) in the absence (A) or in the presence (B) of 10^{-6} M naltrindol (NAL). Two control responses were obtained and DPDPE and NAL were then added as indicated by the dots. Panel C shows the averaged responses (mean ± S.E.M.) from 5–6 experiments such as those shown in panels A and B. $^{**}P < 0.01$.

induced by 10^{-9} M noradrenaline when added either before or during the relaxing phase of this contraction.

4. Discussion

We have analyzed the effects of opioid receptor agonists and antagonists in rat isolated aortic strips. The results can be summarized as follows. (1) Morphine, DPDPE and bremazocine had no effect on resting tension. In contrast, DAMGO induced a contractile response in resting aortic strips which remained after removal of endothelium or in the absence of extracelullar Ca^{2+}. (2) Pretreatment with morphine or bremazocine had no effect on the phasic contractile responses induced by low concentrations (10^{-9} M) of noradrenaline, whereas pretreatment with DPDPE potentiated this phasic contractile response. (3) Morphine and DAMGO, but not DPDPE, added during the relaxation of the phasic contractile responses induced by noradrenaline produced a concentration-dependent contractile response. (4) The contractile responses induced by morphine, DAMGO and DPDPE were blocked by naloxone, β-flunaltrexamine and natrindol, respectively. This indicated that these responses were opioid receptor-mediated and not a non-specific action.

Opioids can play a role in regulating vascular tone either via specific presynaptic opioid receptors or postsynaptic opioid receptors in the vessel wall (Altura et al., 1984; Holaday, 1983; Feuerstein and Siren, 1987). Because of its sparse or absent innervation (Webb et al., 1983), isolated rat aorta was used in the present experiments on the assumption that this preparation would allow us to study the effects of opioid receptor agonists and antagonists directly on vascular smooth muscle, i.e. with minimum interference of prejunctional nerves. Therefore, the present results can only be explained by binding of opioids to extraneuronal sites.

Morphine, DPDPE and bremazocine had no effect on resting tension. Likewise, Lee and Berkowitz (1976) observed that morphine increased the resting tension in rat aorta only at concentrations $\geq 10^{-5}$ M. In contrast, DAMGO produced a concentration-dependent contraction in resting aortic strips which was inhibited by β-flunaltrexamine. The increase in basal tension induced by DAMGO remained unaltered in aortic strips incubated in Ca^{2+}-free PSS, indicating that this contractile response depends on the release of Ca^{2+} from an intracellular source. Furthermore, the presence of a functional endothelium was not required for the contractile response suggesting a direct interaction of DAMGO with the vascular smooth muscle cells. Likewise, Altura et al. (1978) have described opioid-mediated endothelium-independent vascular contractions.

Pretreatment with morphine had no effect on the contractions induced by noradrenaline. However, pretreatment with DPDPE increased the amplitude of the phasic responses induced by noradrenaline, this effect being blocked by naltrindol. The contractile response induced by low concentrations of noradrenaline seems to be due to Ca^{2+} entry, not to intracellular Ca^{2+} release (Van Breemen et al., 1981). Thus, stimulation of δ-opioid receptors might increase Ca^{2+} entry in vascular smooth muscle cells. In bovine aortic smooth muscle, methionine-5-enkephalin (a δ-opioid receptor agonist), but not morphine, increased Ca^{2+} influx and this effect was inhibited by naloxone (Kokkas et al., 1991).

Morphine added when the relaxation of the phasic contraction induced by low concentrations of noradrenaline had reached about 50% of its peak amplitude, induced a contraction which reached values above the peak response to noradrenaline. In contrast, morphine (up to 10^{-6} M) had no effect on the sustained

contractions induced by a maximally effective concentration of noradrenaline (10^{-6} M). Morphine-induced contractile responses were blocked by naloxone, which demonstrated that the effect is an opioid-receptor mediated and not a non-specific action. To determine the specific receptors involved in this contractile response, specific opioid receptor agonists and antagonists were tested. DAMGO also produced a contractile response which was inhibited by β-flunaltrexamine, whereas DPDPE and bremazocine did not elicit a contractile response when added during the relaxing phase of noradrenaline-induced phasic contractions.

These results suggest that stimulation of μ-opioid receptors may directly increase vascular smooth muscle tone, while stimulation of δ- or μ-opioid receptors might act as a modulator of noradrenaline-induced responses. In contrast, stimulation of κ-opioid receptors had no effect on vascular smooth muscle tone. Opioid receptors, apparently of the μ type, have been demonstrated at postsynaptic sites in the cerebral (Hanko and Hardebo, 1978) and mesenteric arteries (Altura et al., 1978). Opioids may be co-stored with noradrenaline in sympathetic nerves (Wilson et al., 1980; Lang et al., 1982). Activation of prejunctional opioid receptors decreases the release of noradrenaline from postganglionic noradrenergic nerve endings innervating the vasculature (Ensinger et al., 1984,1986; Illes et al., 1985). Thus, endogenous opioids could serve as modulators of vasomotor tone during sympathetic activity both at presynaptic and postsynaptic sites.

Unfortunately, the possible role of these direct vascular effects on the overall pressor response produced by DAMGO and (D-Ala^2,D-Leu^5)enkephalin (a δ-opioid receptor agonist) in conscious and anesthetized rats (Pfeiffer et al., 1983; Feuerstein et al., 1983) is unknown. Opioids have complex effects on circulation, so that central nervous and peripheral vascular actions contribute to the overall response. Moreover, in vitro, the vascular effects depend on the opioid and the concentration used, the animal species, the blood vessel and the agonist used (El-Sharkawy et al., 1991; Feuerstein and Siren, 1987). Thus, opioids can contract rat aortic strips (Lee and Berkowitz, 1976; Altura et al., 1978), whereas mesenteric arterioles from the same species respond with vasorelaxation and no response was observed in muscular venules (Altura et al., 1978). Morphine causes a contraction in rat (Waters and Harder, 1983) and canine cerebral arteries (Altura et al., 1984), whereas it relaxes cat middle cerebral arteries (Harder and Madden, 1984).

In conclusion, the present results demonstrated that opioids exert direct effects on vascular smooth muscle tone. At low concentrations, agonists stimulating μ- or δ-opioid receptors seem to act as modulators of the noradrenaline-induced response whereas, at higher concentrations, μ-opioid receptor stimulation may have a direct contractile effect on vascular smooth muscle.

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