the endothelium-dependent vasodilator activity in rats.

**THE ENDONOGUS opioid peptides have been shown to have cardiovascular actions in a variety of species (2–4, 6, 8, 9, 14, 15). However, many of the endogenous opioid peptides have activity that is relatively nonspecific in nature (6, 8, 9). It is known that the endorphins exhibit preferential affinity for the δ-opioid receptor, and the dynorphins bind κ-opioid receptors with high affinity (6, 8, 9). However, it was not until recently that an endogenous μ-selective ligand was isolated (16). Receptor-binding studies indicate that endorphins 1 and 2 are endogenous, potent, selective μ-opioid receptor agonists (16). Endorphin 1 (Tyr-Pro-Trp-Phe-NH₂) and endorphin 2 (Tyr-Pro-Phe-NH₂) possess similar analgesic activity and have recently been shown to decrease systemic arterial pressure (3, 16). A relationship between opioid receptor activation and the release of nitric oxide (NO) has been observed (4, 5, 7, 12, 13, 15). Human arterial and rat microvascular endothelial cells have μ-opioid receptors and release NO when exposed to morphine (15). In isolated rat aorta, morphine-induced vasorelaxant responses are mediated by the release of NO, and the response is attenuated by the opioid receptor antagonist naloxone (15). It has been reported that the μ-selective opioid receptor agonist Tyr-D-Ala-Gly-N-Me-Phe-Gly-ol (DAMGO) did not relax rat aorta, whereas other studies have shown concentration-dependent contractile responses to this synthetic opioid peptide (14, 15). On the basis of previous structure-activity relationships with the opioid peptides, an analog of endorphin 2, D-[Ala²]endomorphin 2 (TAPP), was synthesized. Although endorphins 1 and 2 have been shown to decrease systemic arterial pressure, little if anything is known about responses to TAPP or the mechanism by which the endorphins and TAPP decrease vascular resistance (3). Therefore, the present study was undertaken to compare responses to TAPP and endorphin 2 and to investigate the role of NO and prostaglandin release, and of ATP-dependent K⁺(KATP) channel activation, in mediating vasodilator responses to these opioid receptor agonists.

**MATERIALS AND METHODS**

Seventy Sprague-Dawley rats weighing 290–380 g were anesthetized with pentobarbital sodium (50 mg/kg). Suplemental doses of pentobarbital sodium were administered intravenously as needed during the course of the experiment to ensure a uniform level of anesthesia. The trachea was cannulated, and the rats breathed spontaneously or were ventilated with a Harvard model 683 rodent ventilator at a tidal volume of 2.5 ml and at a rate of 30 breaths/min with room air enriched with 95% O₂-5% CO₂. Catheters were inserted into the external jugular vein for the intravenous administration of drugs and into the carotid artery for the measurement of systemic arterial pressure. For constant-flow perfusion of the hindquarter vascular bed, a 1.0- to 1.5-cm segment of the distal aorta was exposed through a ventral midline incision and cleared of surrounding connective tissue. After administration of heparin sodium (1,000 U/kg iv), the aorta was ligated, and catheters were inserted into the aorta both proximal and distal to the ligature. Blood was withdrawn from the proximal catheter and pumped at a constant flow rate with a Cole-Parmar Masterflex pump into the distal catheter. Perfusion pressure was monitored from a lateral tap in the perfusion circuit located between the pump and the distal aortic catheter. Agonists were injected directly into the hindquarter perfusion circuit distal to the pump in small volumes (30–100 µl) in a random sequence. Hindquarter perfusion circuit distal to the pump in small volumes (30–100 µl) in a random sequence.

**Dependent vasodilator activity in rats.** Am. J. Physiol.
perfusion and systemic arterial pressures were measured with Viggo-Spectramed transducers and were recorded on a Grass model 7 polygraph. Mean pressures were derived from the pulsatile signals by electronic averaging. Hindquarter blood flow was set to achieve a baseline perfusion pressure of \( \sim 125 \) mmHg and was not changed during the remainder of an experiment. The flow rate was determined by timed collection at the conclusion of each experiment and ranged from 5.0 to 7.0 ml/min. The hindquarter vascular bed was surgically denervated by ligating and cutting the lumbar sympathetic chain ganglia between \( L_2 \) and \( L_4 \), as described previously (1). The extent of denervation was assessed by measuring reflex changes in hindquarter perfusion pressure induced by increasing systemic arterial pressure by the intravenous injection of phenylephrine. In the innervated hindquarter vascular bed, the increase in systemic arterial pressure induced by intravenous injection of phenylephrine is associated with a reflex decrease in hindquarter perfusion pressure. After the denervation procedure, the reflex decrease in hindquarter perfusion pressure was greatly attenuated or abolished. Arterial blood gases were measured periodically and were within the physiological range. \( \text{PO}_2, \text{P}_\text{CO}_2, \text{pH} \) were 124 ± 6, 28 ± 2, and 7.39 ± 0.06, respectively, in the control period and did not change during the experimental protocol.

In experiments in which cardiac output was measured, catheters were inserted into the left external jugular vein for the intravenous administration of drugs and into the femoral artery for the measurement of systemic arterial pressure. Cardiac output was measured by the thermodilution technique using a Cardiotherm 500 cardiac output computer (Columbus Instruments, Columbus, OH) equipped with a small animal interface. The thermistor microprobe catheter (Fr-1; Columbus Instruments) was inserted into the right carotid artery and advanced to the aortic arch. A catheter placed in the left jugular vein was advanced to the right atrium for rapid bolus injection of 100 µl (plus catheter dead space) of 10–15°C saline. The saline solution was injected with a Hamilton constant rate syringe to ensure rapid and repeatable injections of the saline indicator solution. Catheter placement was verified by postmortem examination. Mean arterial pressure and heart rate, measured with a Grass model 7P44 tachygraph, were monitored continuously; and cardiac output determinations were made before injection of the agonists, again when arterial pressure decreased to a steady level, and after arterial pressure had returned to the baseline value. These procedures have been described previously (2).

In the first set of experiments, responses to intravenous injections of TAPP, endomorphin 2, nociceptin, and Met-enkephalin were compared; and, in the second set of experiments, the effects of intravenous injections of the opioid peptides on cardiac output, heart rate, and total peripheral resistance were investigated. In the third set of experiments, responses to TAPP and endomorphin 2 were compared and investigated in the hindquarter vascular bed under constant flow conditions. The time course of the decrease in hindquarter perfusion pressure in response to TAPP and endomorphin 2 was compared, and response duration was assessed by comparing the time required for the response to return to 50% of the maximal value \( (t_{50}) \). In the fourth set of experiments, the effects of naloxone on responses to endomorphin 2, TAPP, and Met-enkephalin were compared. Responses were compared before and after administration of naloxone in a dose of 2 mg/kg iv, and the opioid receptor antagonist did not alter systemic arterial or hindquarter perfusion pressure when injected in a dose of 2 mg/kg iv. In the fifth set of experiments, the role of NO release in mediating hindquarter vasodilator responses to TAPP, endomorphin 2, and Met-enkephalin was determined. Responses were compared before and after administration of \( L\)-NAME in a dose of 50 mg/kg iv, and the NO synthase inhibitor increased systemic arterial and hindquarter perfusion pressure from 134 ± 7 to 183 ± 9 mmHg and from 128 ± 11 to 201 ± 13 mmHg, respectively. In the sixth series of experiments, the role of prostaglandin release in mediating vasodilator responses to TAPP and endomorphin 2 was investigated, and vasodilator responses to TAPP, endomorphin 2, and arachidonic acid were compared before and after beginning 15 min after administration of the cyclooxygenase inhibitor sodium mephenesin in a dose of 2.5 mg/kg iv. Administration of sodium mephenesin in a dose of 2.5 mg/kg iv did not alter systemic arterial or hindquarter perfusion pressure. In the last series of experiments, the role of \( K_{\text{ATP}} \) channel activation in mediating vasodilator responses to endomorphin 2 and TAPP was investigated in the hindquarter vascular bed, and vasodilator responses to TAPP, endomorphin 2, and the \( K_{\text{ATP}} \) channel opener levorphanal were compared before and beginning 15 min after administration of the \( K_{\text{ATP}} \) channel antagonist U-37883A in a dose of 5 mg/kg iv. Administration of U-37883A (5 mg/kg iv) did not alter systemic arterial or hindquarter perfusion pressure. In the last set of experiments, a 30-s time-delay coil in the perfusion circuit upstream from the perfusion pump and site of peptide injection was used to determine if responses to TAPP and endomorphin 2 were mediated within the hindquarter vascular bed or if recirculation of the peptides and other humoral factors contributed to the response.

Endomorphin 2 (Ref. 16; synthesized by solution-phase methods or Biochemistry Core, Tulane Medical School, New Orleans, LA), TAPP, diethylamine/NO complex (DEA/NO; Research Biochemicals, Natick, MA), human synthetic adrenomedullin, vasoactive intestinal polypeptide (VIP; Peptide Research Labs, Tulane University School of Medicine, New Orleans, LA), nociceptin (Orphanin FQ), Met-enkephalin (Phoenix Pharmaceuticals, Mountain View, CA), acetylcholine chloride, ATP, isoproterenol hydrochloride, bradykinin, sodium arachidonate, and calcitonin gene-related peptide (CGRP; Sigma Chemical, St. Louis, MO) were dissolved in 0.9% NaCl. Prostaglandin \( E_2 \) (PGE\(_2\); Upjohn, Kalamazoo, MI) was dissolved in 100% ethanol and diluted with 0.9% NaCl. Levorphanal (SmithKline Beecham, Sussex, UK) was dissolved in 0% ethanol-saline solution at a concentration of 10 mg/ml and was diluted with 0.9% NaCl. Drug solutions were
stored frozen in amber bottles. During an experiment, the agonist solutions were kept on crushed ice. The agonists were injected intra-arterially in small volumes (30–100 µl) over a 10- to 15-s period in a random sequence. Sodium meclofenamate, L-NAME (Sigma), and naloxone hydrochloride (Du Pont Pharmaceuticals, Wilmington, DE) were dissolved in 0.9% NaCl. U-37883A (Upjohn) was dissolved in 0.9% NaCl with sonication. The peptides used in these studies were dissolved immediately before use and injected intravenously. Control injections of the vehicles for the agonists and antagonists had no significant effect on systemic arterial or hindquarter perfusion pressure or on responses to the vasoactive agonists. Responses were analyzed using a one-way ANOVA and Scheffe's F-test with a Bonferroni/Dunn procedure or a paired t-test. Baseline values for systemic arterial pressure, heart rate, cardiac output, and total peripheral resistance were 127 ± 7 mmHg, 392 ± 11 beats/min, 124 ± 7 ml/min, and 1.05 ± 0.13 mmHg·ml⁻¹·min, respectively. L-NAME produced a significant increase in hindquarter perfusion pressure, and responses were expressed as percent decrease from baseline to take the increase in baseline perfusion pressure into account. A P value of <0.05 was used as the criterion for statistical significance.

RESULTS

Responses to TAPP and endomorphin 2 in the systemic vascular bed. The effects of intravenous injections of endomorphin 2, TAPP, Met-enkephalin, and nociceptin on systemic arterial pressure were compared in the rat, and these results are summarized in Fig. 1. Endomorphin 2, TAPP, Met-enkephalin, and nociceptin decreased systemic arterial pressure in a dose-related manner when injected in doses of 1–300 nmol/kg iv (Fig. 1). In terms of relative vasodepressor activity, the dose-response curves for endomorphin 2, TAPP, and nociceptin were similar and were approximately one log unit to the left of the dose-response curve for Met-enkephalin when doses of the peptides were compared on a nanomole basis to take molecular weight into account (Fig. 1).
The effects of TAPP and endomorphin 2 on systemic arterial pressure, cardiac output, heart rate, and total peripheral resistance were compared in the rat, and these results are summarized in Fig. 2. Intravenous injections of TAPP and endomorphin 2 in doses of 30 and 100 nmol/kg produced similar dose-related decreases in systemic arterial pressure and total peripheral resistance (Fig. 2). Heart rate and cardiac output were decreased significantly at each dose of endomorphin 2 and the endomorphin 2 analog studied (Fig. 2).

Responses to TAPP and endomorphin 2 in the hindquarter vascular bed. Regional vascular responses to TAPP and endomorphin 2 were compared under constant-flow conditions, and injections of TAPP and endomorphin 2 into the perfusion circuit in doses of 3–100 nmol produced dose-related decreases in hindquarter perfusion pressure. In terms of relative vasodilator activity, responses to TAPP and endomorphin 2 were similar in magnitude when doses of the peptides were compared on nanomole basis; and bradykinin and VIP were ~30- to 100-fold more potent than TAPP or endomorphin 2 in decreasing hindquarter perfusion pressure in the rat (data not shown).

The time courses of the decreases in hindquarter perfusion pressure in response to the 100-nmol dose of TAPP and endomorphin 2 are shown in Fig. 3. Vasodilator responses to these peptides were rapid in onset, with perfusion pressure falling to a nadir ~30 s after injection (Fig. 3). However, the duration of the decrease in perfusion pressure in response to TAPP was significantly longer than the duration of the response to endomorphin 2 (Fig. 3). The time to peak vasodilator response to TAPP and endomorphin 2 was compared in Fig. 3. The time of the vasodilator response to TAPP was significantly longer than the time of vasodilator responses to endomorphin 2 when the peptides were injected in doses of 30 and 100 nmol (Fig. 3).

Influence of naloxone. The influence of the opioid receptor antagonist naloxone on vasodilator responses to TAPP, 2, and Met-enkephalin was compared, and these data are summarized in Fig. 4. After administration of naloxone in a dose of 2 mg/kg iv, decreases in hindquarter perfusion pressure in response to TAPP, endomorphin 2, and Met-enkephalin were significantly reduced (Fig. 4). In contrast, vasodilator responses to the opioid receptor-like (ORL1) receptor ligand nociceptin, DEAN/NO, isoproteol, and ATP were not altered after administration of the opioid receptor antagonist (Fig. 4).

Influence of L-NAME. The role of nitric oxide release in mediating vasodilator responses to TAPP and endomorphin 2 was investigated, and these results are summarized in Fig. 5. After administration of the NO synthase inhibitor L-NAME in a dose of 50 mg/kg iv, decreases in hindquarter perfusion pressure in response to endomorphin 2, TAPP, and Met-enkephalin were significantly reduced (Fig. 5). Hindquarter vasodilator responses to acetylcholine and adrenomedullin were also reduced significantly after administration of L-NAME (Fig. 5). In contrast to the effects of L-NAME on responses to TAPP, endomorphin 2, acetylcholine,
and adrenomedullin, vasodilator responses to CGRP, isoproterenol, levcromakalim were not altered after administration of the nitric oxide synthase inhibitor (Fig. 6).

Effects of sodium meclofenamate, U-37883A, and a time-delay coil. The effects of the cyclooxygenase inhibitor sodium meclofenamate, the K$_{ATP}$ channel antagonist U-37883A, and the presence of a time-delay coil in the perfusion circuit on responses to TAPP and endomorphin 2 were investigated, and these data are summarized in Fig. 7 and Table 1. Hindquarter vasodilator responses to TAPP and endomorphin 2 were not altered by 2.5 mg/kg iv sodium meclofenamate, a dose that attenuated vasodilator responses to the prostaglandin precursor arachidonic acid (Fig. 7). Hindquarter vasodilator responses to TAPP and endomorphin 2 were not altered by U-37883A in a dose of 5 mg/kg iv (Fig. 8). Vasodilator responses to the K$_{ATP}$ channel opener levcromakalim were significantly attenuated by the K$_{ATP}$ channel antagonist U-37883A (Fig. 7). The presence of a 30-s time-delay coil in the perfusion circuit upstream from the perfusion pump and the site of peptide injection had no significant effect on the magnitude or the time course of the decrease in hindquarter perfusion pressure in response to TAPP and endomorphin 2 when injected into the perfusion circuit in a dose of 100 nmol (Table 1).

**DISCUSSION**

The endomorphins are recently discovered endogenous agonists reported to be selective for the µ-opioid receptor (16). In addition to possessing analgesic activity, it recently has been shown that these novel peptides decrease systemic arterial pressure (3). The results of the present study extend this finding by showing that the endomorphin 2 analog TAPP decreases systemic arterial pressure in a manner similar to endomorphin 2 and was ~10-fold more potent than Met-enkephalin. Moreover, the results of the present study show that decreases in systemic arterial pressure in response to TAPP and endomorphin 2 are associated with decreases in cardiac output, heart rate, and total peripheral resistance and that responses to both peptides are similar. These data indicate that the effects of the opioid receptor agonists on the total peripheral resistance are greater than the effects on cardiac output and suggest that TAPP and endomorphin 2 have vasodilator activity in the systemic vascular bed of the rat. The mechanism of the decreases in cardiac output associated with injection of TAPP and endomorphin 2 is uncertain but may be related to the decrease in heart rate. This decrease in cardiac output and heart rate may be due, in part, to a central nervous system effect of the peptide, to an effect on the heart, or a direct negative inotropic effect on the myocardium. The direct
The effects of TAPP and endomorphin 2 on myocardial function in the rat should be investigated in future studies. Although the mechanism mediating the inappropriate decrease in heart rate associated with the decrease in arterial pressure in response to TAPP and endomorphin 2 is unknown, these results are similar to studies with nociceptin showing that decreases in systemic arterial pressure and total peripheral resistance in response to the ORL1 ligand are associated with decreases in cardiac output and heart rate in the rat (2).

Because intravenous injections of TAPP and endomorphin 2 produced changes in pressure and cardiac output, complicating the interpretation of hemodynamic responses to the peptides, the effects of TAPP and endomorphin 2 on regional vascular resistance were further studied in the hindquarter vascular bed of the rat under constant-flow conditions, where injections of TAPP and endomorphin 2 into the perfusion circuit induced similar dose-related decreases in hindquarter perfusion pressure. However, the opioids were far less potent than bradykinin and VIP in dilating the hindquarter vascular bed. TAPP and endomorphin 2 were ∼10-fold more potent than Met-enkephalin in the hindquarter and systemic vascular bed of the rat but had similar vasodepressor activity in the rabbit, suggesting a species difference in relative potency (3). Inasmuch as blood flow was maintained constant, the decreases in perfusion pressure in response to the peptides reflect decreases in hindquarter vascular resistance. The vasodilator responses to TAPP and endomorphin 2 were independent of an action on the adrenergic nervous system, since the hindquarter vascular bed was surgically denervated. The results showing that vasodilator responses to TAPP and endomorphin 2 were not changed in magnitude or in time course by the imposition of a 30-s time-delay coil in the perfusion circuit upstream from the pump and from the site of peptide injection suggest that responses to these peptides are mediated locally within the hindquarter vascular bed. The observation that TAPP and endomorphin 2 have similar vasodilator activity suggests that substitution of the proline residue with D-alanine in the linear sequence of endomorphin 2 does not alter the vasodilator activity of the peptide. Although the magnitude of vasodilator responses to TAPP and endomorphin 2 was similar, the duration of the response to TAPP, as measured by the response recovery t½, was significantly longer than the response to endomorphin 2 when the 30- and 100-nmol doses were compared. It has been suggested that substitution of the proline in the second position with D-alanine residue may inhibit proteolytic cleavage of the peptide, thereby increasing potency and/or duration of action. The results of the present study showing that the magnitude of the vasodilator response is not altered but that response duration is increased with the higher doses of the analog studied may be interpreted to suggest that the D-alanine substitution inhibits the degradation of the peptide by proteolytic processing and increases the duration of action in the hindquarter vascular bed of the rat.

The relative magnitude of the vasodilator response to the 30-nmol dose of TAPP and endomorphin 2, which was an ∼20–30% decrease in vascular resistance in the hindquarter vascular bed, and the decrease in total peripheral resistance, which was ∼30% at the 100 nmol/kg iv dose of the peptides, were approximately similar when dilution of the peptides in the circulation was taken into account. These data suggest that the relative vasodilator activities of TAPP and endomorphin 2 were approximately similar in the hindquarter vascular bed and in the systemic vascular bed of the rat.

The effect of the opioid receptor antagonist naloxone on vasodilator responses to TAPP was investigated, and responses to TAPP, endomorphin 2, and Met-enkephalin were attenuated by naloxone. The inhibitory effects of naloxone were selective in that vasodilator responses to TAPP and endomorphin 2 were independent of an action on the adrenergic nervous system, since the hindquarter vascular bed was surgically denervated. The results showing that vasodilator responses to TAPP and endomorphin 2 were not changed in magnitude or in time course by the imposition of a 30-s time-delay coil in the perfusion circuit upstream from the pump and from the site of peptide injection suggest that responses to these peptides are mediated locally within the hindquarter vascular bed. The observation that TAPP and endomorphin 2 have similar vasodilator activity suggests that substitution of the proline residue with D-alanine in the linear sequence of endomorphin 2 does not alter the vasodilator activity of the peptide. Although the magnitude of vasodilator responses to TAPP and endomorphin 2 was similar, the duration of the response to TAPP, as measured by the response recovery t½, was significantly longer than the response to endomorphin 2 when the 30- and 100-nmol doses were compared. It has been suggested that substitution of the proline in the second position with D-alanine residue may inhibit proteolytic cleavage of the peptide, thereby increasing potency and/or duration of action. The results of the present study showing that the magnitude of the vasodilator response is not altered but that response duration is increased with the higher doses of the analog studied may be interpreted to suggest that the D-alanine substitution inhibits the degradation of the peptide by proteolytic processing and increases the duration of action in the hindquarter vascular bed of the rat.

The relative magnitude of the vasodilator response to the 30-nmol dose of TAPP and endomorphin 2, which was an ∼20–30% decrease in vascular resistance in the hindquarter vascular bed, and the decrease in total peripheral resistance, which was ∼30% at the 100 nmol/kg iv dose of the peptides, were approximately similar when dilution of the peptides in the circulation was taken into account. These data suggest that the relative vasodilator activities of TAPP and endomorphin 2 were approximately similar in the hindquarter vascular bed and in the systemic vascular bed of the rat.

The effect of the opioid receptor antagonist naloxone on vasodilator responses to TAPP was investigated, and responses to TAPP, endomorphin 2, and Met-enkephalin were attenuated by naloxone. The inhibitory effects of naloxone were selective in that vasodilatation...
tor responses to nociceptin, DEA/NO, isoproterenol, and ATP were not altered by the opioid receptor antagonist. These data suggest that hindquarter vasodilator responses to TAPP, endomorphin 2, and Met-enkephalin are mediated by a naloxone-sensitive mechanism. It has been reported that µ-opioid receptors are present on endothelial cells and that morphine has vasorelaxant activity mediated by the release of NO from the endothelium (15). It has also been reported that the synthetic µ-opioid receptor agonist DAMGO induced vasodilation of rat pial arteries mediated by the release of NO (4). It was reported, however, that DAMGO did not relax vascular smooth muscle or release NO and that this peptide induced concentration-dependent contractile responses in rat aortic strips (14, 15). The differences in response to DAMGO may involve differences in vascular preparation studied, and the results of preliminary studies in the rat hindquarter vascular bed show that DAMGO induces dose-related decreases in perfusion pressure that are attenuated by L-NAME. The results of preliminary studies in the rat hindquarter vascular bed with DAMGO are consistent with results in rat pial arteries (4).

In the present study, the effects of the NO synthase inhibitor L-NAME on vasodilator responses to TAPP, endomorphin 2, and Met-enkephalin were investigated, and these results show that vasodilator responses to the three peptides were significantly decreased by L-NAME in a dose that attenuated responses to the endothelium-dependent vasodilator agents acetylcholine and adrenomedullin. The observation that vasodilator responses to TAPP, endomorphin 2, acetylcholine, and adrenomedullin were attenuated by L-NAME, whereas vasodilator responses to the NO donor DEA/NO to isoproterenol, levcromakalim, and to CGRP were not altered, provides support for the hypothesis that vasodilator responses to TAPP, endomorphin 2, and Met-enkephalin are mediated at least in part by the release of NO from the endothelium and is consistent with results showing that vasodilator responses to DAMGO are attenuated by inhibitors of NO synthesis in rat pial arteries (4).

The effects of the cyclooxygenase inhibitor sodium (1R,2R)-N-methyl-L-argenofenate and of the K<sub>ATP</sub> channel antagonist U-37883A on vasodilator responses to TAPP and endomorphin 2 were investigated, and these results show that vasodilator responses are not altered by sodium (1R,2R)-N-methyl-L-argenofenate in a dose that attenuated vasodilator responses to the prostaglandin precursor arachidonic acid or U-37883A in a dose that reduced vasodilator responses to the K<sub>ATP</sub> channel opener levcromakalim. These data suggest that the release of vasodilator prostaglandins or the opening of K<sub>ATP</sub> channels is not involved in mediating vasodilator responses to endomorphin 2 or TAPP in the hindquarter vascular bed of the rat. It has been reported that L-arginine analogs inhibit vasodilator responses to the K<sub>ATP</sub> channel openers in rat pial vessels (11). To determine if the L-arginine analog L-NAME altered vasodilator responses to the K<sub>ATP</sub> channel agonists in the hindquarter vascular bed of the rat, the effects of the NO synthase inhibitor on responses to levcromakalim were investigated. The results of these studies show that vasodilator responses to levcromakalim are not altered by L-NAME in a dose that attenuated responses to the endothelium-dependent vasodilator agents but are reduced by the K<sub>ATP</sub> channel antagonist U-37883A, suggesting that this L-arginine analog did not block K<sub>ATP</sub> channels in the hindquarter vascular bed in the dose used. The reason for the difference in the effects of NO synthase inhibitors on vasodilator responses to K<sub>ATP</sub> channel agonists in rat pial vessels and in the hindquarter vascular bed of the rat is uncertain but may involve regional differences in the vascular bed studied or experimental procedure employed. It has also been shown that L-arginine analogs inhibit responses to the vasodilator prostaglandins (10). However, in the present study in the hindquarter vascular bed, L-NAME in a dose that attenuated responses to TAPP, endomorphin 2, Met-enkephalin, acetylcholine, and adrenomedullin did not alter vasodilator responses to PGE<sub>1</sub>. These data suggest that an interaction between L-NAME and vasodilator prostaglandin receptors is unlikely in the hindquarter vascular bed of the rat.

In summary, the results of the present study show that TAPP has vasodilator activity similar to endomorphin 2 in the rat. Decreases in systemic arterial pressure in response to TAPP are associated with decreases in heart rate, cardiac output, and total peripheral resistance similar to responses elicited by endomorphin 2. In the hindquarter vascular bed under constant flow conditions, TAPP and endomorphin 2 produced similar dose-dependent decreases in hindquarter perfusion pressure. Vasodilator responses to TAPP and endomorphin 2 in the hindquarter vascular bed were attenuated by the opioid receptor antagonist naloxone and by the NO synthase inhibitor L-NAME but not by the K<sub>ATP</sub> Channel antagonist U-37883A or the cyclooxygenase inhibitor sodium medofenate. Hindquarter vasodilator responses to TAPP and endomorphin 2 were not altered by the presence of a 30-s time delay coil in the perfusion circuit. These results indicate that vasodilator responses to TAPP and endomorphin 2 are mediated by an NO-dependent naloxone-sensitive mechanism within the hindquarter vascular bed of the rat.

We thank J anice Ignarro for editorial assistance.

The studies were supported, in part, by National Heart, Lung, and Blood Institute (NHLBI) Grant HL-15580 and by a grant from the American Heart Association-Louisiana. H. C. Champion was supported by NHLBI Grant HL-9474.

Address for reprint requests: P. J. Kadowitz, Dept. of Pharmacology SL83, Tulane University School of Medicine, 1430 Tulane Ave., New Orleans, LA 70112.

Received 26 September 1997; accepted in final form 14 January 1998.

REFERENCES

1. Champion, H. C., M. A. Czapla, D. E. Friedman, D. G. Lambert, W. A. Murphy, D. H. Coy, and P. J. Kadowitz. Nociceptin, an endogenous ligand for the ORL<sub>1</sub> receptor, decreases...


