Decreased Vascular Resistance after Intra-Arterial Injection of [Met]enkephalin in the Hindquarters of Conscious Rabbits[1]

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ABSTRACT

The hemodynamic effects of i.a. [met]enkephalin were studied in the hindquarter vasculature of chronically prepared, conscious rabbits. A new method allowed i.a. injection while simultaneously measuring blood pressure and blood flow to this vascular bed. [Met]enkephalin produced a dose-dependent (3–300 μg/kg) decrease in hindquarter vascular resistance (18–42% change from base line). The duration of the response ranged from 28 sec to over 2 min. Heart rate decreased 16 to 45% over the same dose range but returned to preinjection levels in 5 sec. Only the bradycardia was abolished by pretreatment with atropine methyl nitrate. All hemodynamic changes were eliminated or significantly reduced after pretreatment with the ganglionic blocking agent, chlorisondamine hydrochloride. The opioid antagonist, naloxone hydrochloride, abolished the hemodynamic effects of [met]enkephalin. Resistance decreases in the mesenteric vasculature were coincident with those in the hindquarters. The time to onset of the response was delayed when [met]enkephalin was injected i.v. These data indicate activation of a reflex originating in the hindquarters that resulted in opioid-dependent increased efferent parasympathetic activity to the heart and decreased sympathetic tone to at least two vascular beds.

One of the endogenous opioid peptides, mENK, is found in adrenal chromaffin cells, where it is costored and coreleased with catecholamines (Viveros et al., 1979). Stimulation of the splanchnic nerve (Hanbauer et al., 1982) or hemorrhagic hypotension (Lang et al., 1982) causes mENK to be released along with catecholamines in anesthetized dogs. In addition, release of this peptide after splanchnic nerve stimulation in reserpinized dogs produces a frequency-dependent hypotension that can be blocked with naloxone (Hanbauer et al., 1982).

In some models, exogenous mENK has been shown to be a hypotensive agent. Intravenous injection of mENK decreases arterial pressure in pentobarbital-anesthetized cats (Moore and Dowling, 1980), rabbits (Rhee et al., 1985) and dogs (Caffrey et al., 1985). Intra-arterial injection of mENK produces dose-dependent decreases in vascular resistance in isolated, nerve-stimulated cat spleens in vitro (Gaddis and Dixon, 1982) and in isolated hindlimbs of anesthetized cats in situ (Moore and Dowling, 1982). The mechanism of the hypotensive effect of mENK appears to vary with the preparation. In the cat spleen, mENK presynaptically inhibits norepinephrine release (Gaddis and Dixon, 1982). Rhee et al. (1985) showed that the hypoten-

sive effect of i.v. mENK in anesthetized rabbits was secondary to decreased sympathetic nerve activity. Ruth et al. (1984) have demonstrated a vasodilatory effect of mENK in isolated rat aorta that was due to a decrease in the response to norepinephrine.

Considering a potential source of mENK (*i.e.*, the adrenal medulla), as well as its hypotensive effects in some models, we considered the possibility that endogenous mENK might act as a hypotensive agent. As a first step, we examined the hemodynamic effects of mENK in an experimental model uncomplicated by anesthesia or recent surgery. We developed a new, chronic preparation that allowed us to make close-arterial injections in the hindquarters of conscious rabbits while simultaneously measuring arterial pressure and blood flow to the region. Our hypothesis was that i.a. mENK would decrease hindquarter vascular resistance.

Methods

Preparation. Twenty-five male New Zealand White rabbits weighing from 2.3 to 3.2 kg ($\bar{x}$ ± S.E. = 2.7 ± 0.0 kg) were chronically prepared during a midline laparotomy. Surgery was successful in 22 rabbits. A mixture of oxygen and halothane (Halocarbon Laboratories, Inc., Hackensack, NJ) was used for anesthesia. The experimental protocol required two arterial and one venous catheter (Gronan et al., 1983) and a 3-mm-inside-diameter Doppler flow probe on the terminal aorta. The preparation is shown in figure 1. The more caudal arterial

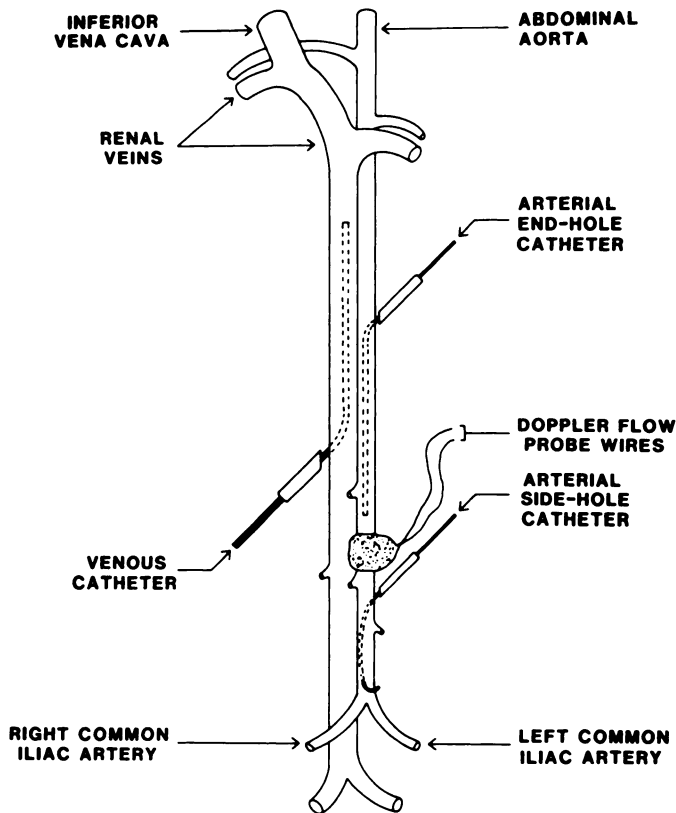**ABBREVIATION:** mENK, [met]enkephalin.

Fig. 1. Surgical preparation of the rabbits. The relationships of the three catheters and the terminal aortic flow probe to the common iliac arteries are shown. All wires and catheters exited the animal at the nape of the neck.

catheter was modified by cutting a hole in the side of the Silastic tubing and filling the remainder with silicone rubber (Dow Corning Corp., Midland, MI). Antibiotics (60,000 U i.m.; Benza-Pen: Beecham Laboratories, Bristol, TN) were administered the day before and the day after surgery.

Catheter patency was maintained by flushing three times weekly with 2 ml of heparinized (10 U/ml) saline solution (0.9% sodium chloride; Travenol Laboratories, Inc., Morton Grove, IL) and filling with 0.5 ml of heparin (1000 U/ml; Elkins-Sinn, Inc., Cherry Hill, NJ). Rabbits were trained in the experimental environment on each of 3 days before the first experiment and on the day before all additional experiments. The first experiment was done at least 10 days after surgery.

General protocol. Animals were fasted for 16 to 20 hr before the experiment. During the experiment, the rabbit was placed in a box that restricted his movement, and saline-filled extensions were connected to the catheters. The animal was heparinized (2000 U of heparin sodium i.v.; Eli Lilly and Co., Indianapolis, IN), the rostral arterial catheter was connected to a pressure transducer (model P23Db; Gould-Statham, Cleveland, OH) and the flow probe wires were connected to a 10-mHz, pulsed-Doppler flowmeter (Hartley and Cole, 1974).

Heart rate, terminal aortic blood flow and mean arterial pressure were monitored on a chart recorder (model 2800; Gould Inc., Cleveland, OH) throughout the experiment. Pulsatile pressure and flow signals were recorded (model 5600c; Honeywell, Littleton, CO) on half-inch magnetic tape (797; Ampex Corp., Redwood City, CA). Solutions of mENK (Tyr-Gly-Gly-Phe-Met; Peninsula Laboratories, Inc., Belmont, CA) were made in sterile saline solution on the morning of the experiment. All other drugs were prepared in a similar manner. Injections of all compounds were made by loading the appropriate catheter with a small volume (0.2 ml/kg b.wt.) of the solution and flushing it into the animal with 2 ml of heparinized saline over 10 sec. Hemodynamic variables were monitored for 3 min postinjection.

Intra-arterial dose-response series. After establishing a base line for control hemodynamic measurements, 0.9% saline or one of six doses (1, 3, 10, 30, 100 or 300 μg/kg) of mENK was injected i.a. through the arterial side-hole catheter. The order of the seven injections was random, and each of the eight rabbits received no more than one dose per day.

Tests of mechanism involved in the hemodynamic response to i.a. mENK. Seven rabbits received mENK (30 μg/kg) through the vena caval catheter (i.v.), as well as i.a. The i.a. and i.v. responses were compared. The general protocol was identical with the dose-response series.

Five rabbits were given the opioid antagonist, naloxone hydrochloride (1 mg/kg i.v.; Endo Laboratories, Inc., Garden City, NY), before mENK (30 μg/kg i.a.). This experiment was compared with the same one done without naloxone pretreatment.

Seven rabbits were given a parasympatholytic dose of atropine methyl nitrate (100 μg/kg i.v.; Sigma Chemical Co., St. Louis, MO). A maximum of 3 min was allowed for the animal to become stable after atropine administration. At the end of this period, mENK (30 μg/kg was injected i.a. The absence of bradycardia in response to acetylcholine chloride (100 μg/kg i.v.; Sigma) given at the conclusion of the experiment was taken as evidence of muscarinic blockade. Similar experiments were done without prior muscarinic blockade.

Blockade of autonomic ganglia was produced in five rabbits by pretreatment with chlorisondamine hydrochloride (2 mg/kg i.v.; Ciba Pharmaceutical Co., Summit, NJ) 1 to 2 hr before the mENK injection (30 μg/kg). To verify ganglionic blockade, a puff of cigarette smoke was blown near the rabbit's nose at the conclusion of the experiment. In untreated rabbits, this causes bradycardia, a pressor response, and a decrease in terminal aortic flow (White et al., 1974b). These changes are mediated by increased cardiac vagal and peripheral sympathetic activity. The absence of these changes was taken as evidence of autonomic blockade. Similar experiments were done in the same rabbits without ganglionic blockade.

Rabbits were not used for another experiment for at least 2 days after receiving atropine or naloxone. Rabbits that received chlorisondamine were not used again for a least 1 week.

Data analysis. Results are reported from 18 of 22 rabbits on which surgery was successful. Technical problems (e.g., catheter failure) prevented experiments in four rabbits. Most of the rabbits were used for more than one experiment. Consecutive experiments on the same rabbit were separated by at least 1 day.

Low-pass filters (0.2 Hz) were used to obtain tracings of mean arterial pressure and flow from the tape-recorded pulsatile signals. The frequency shift in the Doppler signal was assumed to be linearly related to flow (White et al., 1974a) and is given in kilohertz. If an aortic diameter of 3 mm is assumed, a frequency shift of 2.0 kHz is equivalent to a flow rate of 90 ml/min. The unfiltered, pulsatile pressure signal was used to trigger a tachograph to provide heart rate. Vascular resistance was calculated by dividing mean flow into mean arterial pressure.

Preinjection values for all parameters were the mean of five consecutive points taken at 10-sec intervals before the beginning of the flush of saline or mENK. The beginning of the flush was taken as time = 0, and data were analyzed every 5 sec for 1 min. Additional points were taken at 7 sec for i.a. experiments and 12 sec for i.v. experiments. All data are shown either as the percent change from control (%Δ) or as the raw values (mean ± 1 S.E). Statistical significance of changes over time after mENK was evaluated by analysis of variance. If a significant F value was found, a least-significant difference test (Snedecor and Cochran, 1967) was used to detect differences between individual means. Statistical significance in the blocking-drug studies was assessed using a paired t test to compare the maximum effects of mENK with and without prior blockade. Throughout the data analysis, significant differences were determined when $P < .05$.

Results

Hemodynamic effects of i.a. injection of mENK in the conscious rabbit. The response to mENK was biphasic (fig. 2, left panel). The initial response consisted of asystole, decreased arterial pressure and decreased terminal aortic blood flow. This response occurred 5 to 7 sec after the beginning of the flush. After this early response, heart rate and blood pressure returned to control levels, and flow increased above control.

The base-line values for the eight animals used in the dose-response studies were: heart rate, 160 ± 7 beats/min; mean arterial pressure, 68 ± 2 mm Hg; and mean terminal aortic blood flow, 2.1 ± 0.1 kHz. Preliminary studies with 0.1, 0.3 and 1.0 μg/kg of mENK indicated that these doses were not different from saline controls. Figure 3 illustrates the dose-related hemodynamic changes after i.a. injection of mENK or saline. All responses to mENK (at doses ≥3 μg/kg) were dose dependent and began 5 ± 0 sec after the beginning of the flush. The heart rate response to 3 μg/kg was a 16% decrease, and the decrease at greater doses exceeded 40% (fig. 3A). Asystole was produced in three rabbits at all doses ≥10 μg/kg. In asystolic rabbits, blood pressure reached a nadir just before the next beat. The magnitude of the pressure decrease, as well as its duration, was dose dependent (fig. 3B). At the highest dose of mENK (300 μg/kg), there was a secondary increase in blood pressure.

Between 5 and 7 sec after the beginning of the flush, there was a sharp decrease in hindquarter blood flow, coincident with the decrease in heart rate (fig. 3C). Less than 5 sec later, flow increased and remained above base line after heart rate and arterial pressure had returned to their control levels. Resistance values were not calculated under conditions of zero flow (*i.e.*, during asystole). This occurred at the 7-sec point for all doses ≥10 μg/kg. The maximum decrease in resistance occurred 15 to 25 sec after the beginning of the flush (fig. 3D). The magnitude and duration of the response were dose-dependent.

Mechanisms involved in the hemodynamic response to i.a. mENK. Figure 4 compares the i.a. and i.v. responses to mENK (30 μg/kg). With i.v. injection, the response began 10 ± 1 sec after the beginning of the flush, 4 to 6 sec later than after i.a. injection. Superimposing the i.a. and i.v. plots so that the onsets were coincident (not shown) revealed that there were no significant differences in the time to maximum effect or the magnitude of the response. However, resistance returned to base line more rapidly after i.v. mENK.

The hemodynamic results of mENK injection (30 μg/kg i.a.) with and without pretreatment with naloxone hydrochloride, atropine methyl nitrate or chlorisondamine hydrochloride are shown in table 1. Control values were taken before mENK and are shown for each set of experiments. Percent changes after mENK were calculated at the points of greatest change in each parameter. These did not necessarily occur at the same time point. Systemic opioid receptor blockade with naloxone hydrochloride (1 mg/kg i.v.) abolished all hemodynamic effects of mENK (fig. 2, right panel; table 1). Muscarinic blockade with atropine methyl nitrate (0.1 mg/kg i.v.) eliminated the bradycardia produced by mENK and significantly reduced the decrease in pressure (table 1). The initial decrease in flow, coincident with the bradycardia, was abolished (data not shown). The delayed increase in hindquarter blood flow and the associated decrease in vascular resistance were still present (table 1). In the absence of bradycardia, these responses began 8 ± 1 sec after the beginning of the flush. Blockade of autonomic ganglia with chlorisondamine hydrochloride (2 mg/kg i.v.) eliminated or significantly reduced all hemodynamic effects of mENK (table 1).

Based on the results to this point, the response to mENK appeared to be mediated at least in part through a central nervous system reflex. In order to test this possibility further, we instrumented six rabbits for simultaneous measurement of
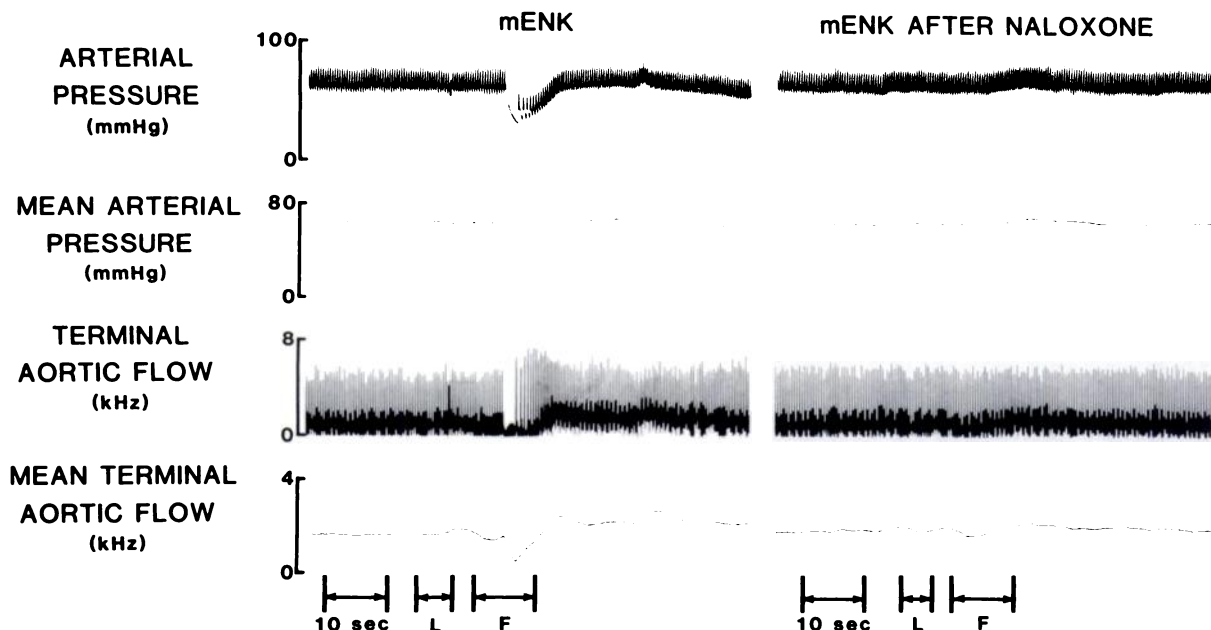

Fig. 2. Hemodynamic changes after i.a. injection of mENK (30 μg/kg) into the hindquarters of a conscious rabbit. The arterial side-hole catheter was loaded with mENK solution at "L" and flushed into the animal over the interval "F." The left panel shows the mENK response with no pretreatment. The right panel shows the response to mENK after pretreatment with naloxone (1 mg/kg i.v.).
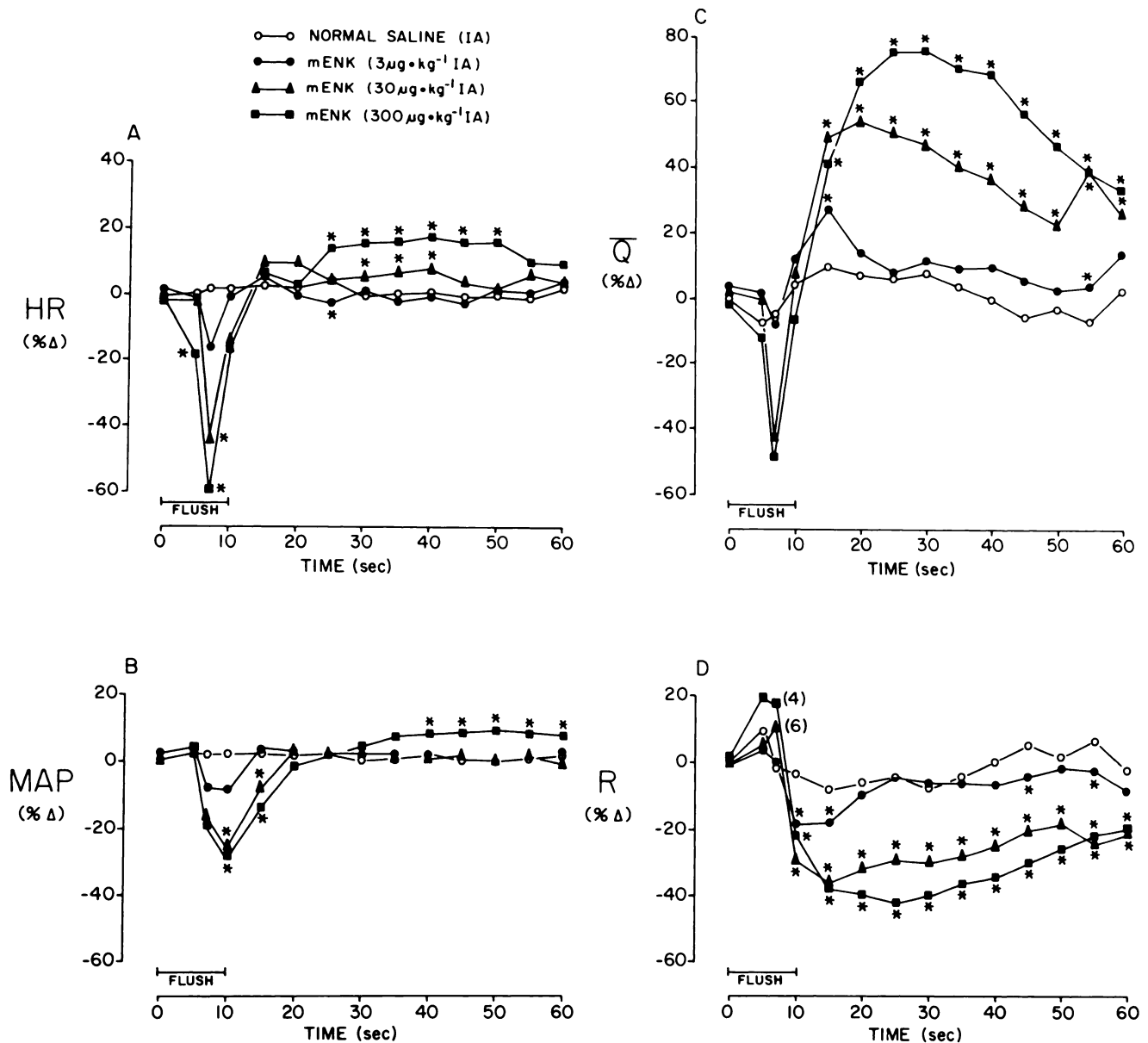
Fig. 3. Dose-dependent changes in heart rate, mean aortic pressure, mean terminal aortic blood flow and hindquarter vascular resistance over time after mENK injection into the terminal aorta of conscious rabbits. The responses are plotted as percent changes (%Δ) from the preinjection value for each parameter. S.E. bars are omitted for clarity. The beginning of the flush was time = 0 for all measurements. The mean response (*n* = 8 for all doses except 300 μg/kg, where *n* = 7) to injection of the vehicle control (0.9% saline) and three doses of mENK, 3, 30 and 300 μg/kg, are shown. * Significantly different (P < .05) from the control, saline-treated group. Doses of 1, 10 and 100 μg/kg are omitted for clarity. Numbers in parentheses indicate the reduced sample size at points where asystole (zero flow) precluded calculation of resistance in some animals. HR, heart rate; MAP, mean aortic pressure; Q̄, mean terminal aortic blood flow; R, hindquarter vascular resistance.

terminal aortic and superior mesenteric artery (an artery proximal to the injection site) blood flow. Figure 5 shows a representative example of one of these experiments. The onset latency of the increase in blood flow in both beds was the same. The magnitude of the response in the superior mesenteric bed was consistently less than in the hindquarters.

Discussion

The endogenous opioid peptides have been the subject of intense investigation focused on the possible role of these compounds in neurohumoral control of the cardiovascular system (Holaday, 1983). It has been shown that β-endorphin is coreleased with adrenocorticotropin from the anterior and in-

termediate pituitary (Guillemin *et al.*, 1977) and that mENK is coreleased with catecholamines from the adrenal medulla (Viveros *et al.*, 1979). Intravenous-endorphin (Lemaire *et al.*, 1978) or mENK (Moore and Dowling, 1980; Caffrey *et al.*, 1985; Rhee *et al.*, 1985) has been reported to cause hypotension. Opioid antagonism with naloxone reverses the hypotension in shock states due to endotoxemia (Holaday and Faden, 1978), hemorrhage (Faden and Holaday, 1979), spinal cord transection (Holaday and Faden, 1980) and anaphylaxis (Amir, 1982). This evidence implicates endogenous opioids in cardiovascular control during various hypotensive states, but the peptide(s) involved and their site(s) of action remain unknown.

We developed a preparation that allowed us to evaluate the hemodynamic effects of mENK in the hindquarter vasculature
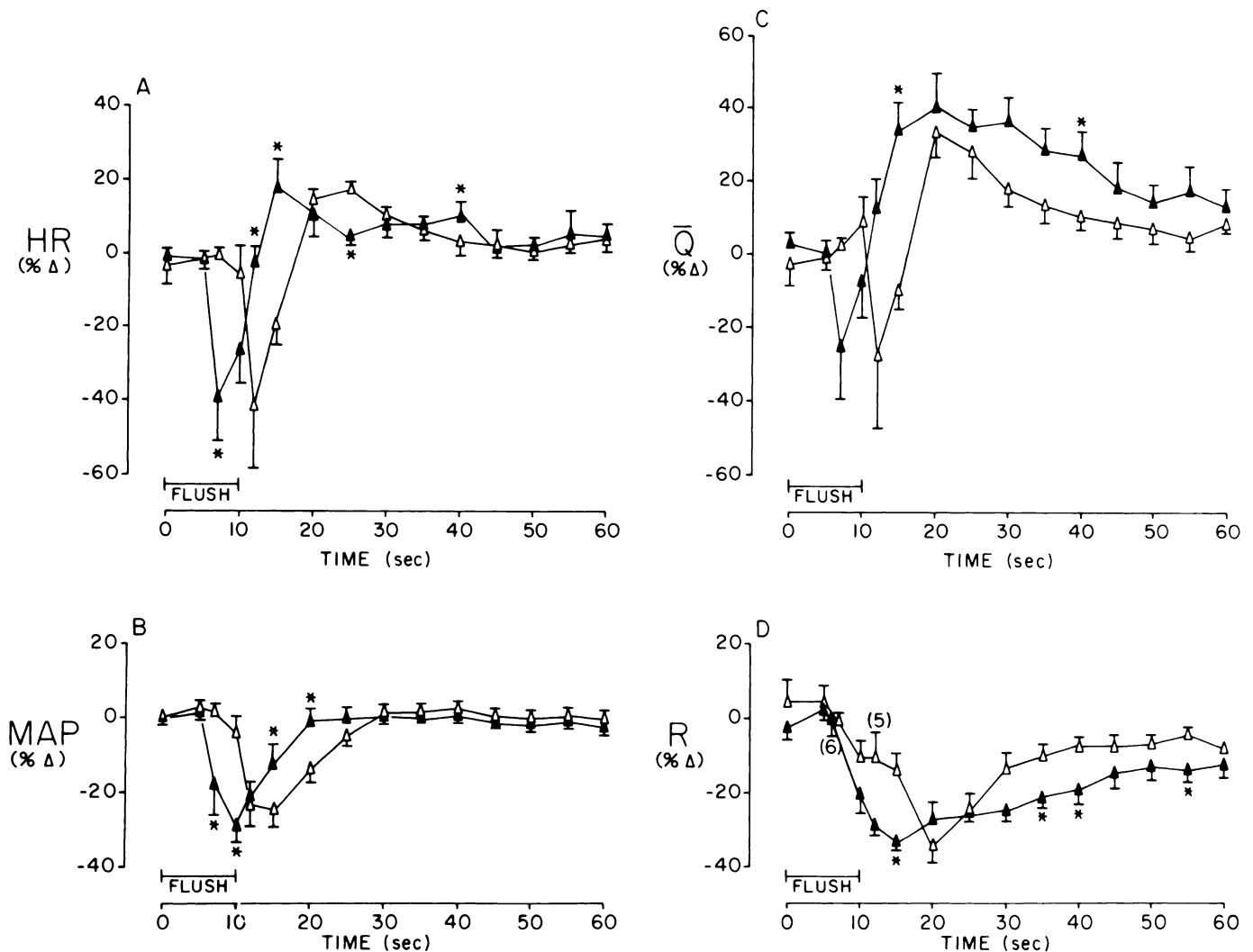
Fig. 4. Comparison of i.a. and i.v. administration of mENK (30 μg/kg). Values are the mean ± 1 S.E. (*n* = 7). Abbreviations are as in figure 3. * P < .05. Numbers in parentheses indicate the reduced sample size at points where asystole (zero flow) precluded calculation of resistance in some animals.

TABLE 1

Effects of opioid, muscarinic and ganglionic blockade on the hemodynamic response to i.a. mENK

Values shown are heart rate, mean arterial blood pressure, mean blood flow in the terminal aorta and terminal aortic vascular resistance and are shown as the mean ± 1 S.E. Raw values before injection of mENK, as well as the maximum change (%Δ) after injection, is shown. The maximum change in response to mENK is represented as the percent change from preinjection ± 1 S.E. The maximum change with blockade is measured at the same time postinjection as without blockade. HR, heart rate; MAP, mean arterial blood pressure; Q̄, mean blood flow in the terminal aorta; R, terminal aortic vascular resistance.

	Naloxone[a]		Atropine[b]		Chlorisondamine[c]	
	Without blockade	With blockade	Without blockade	With blockade	Without blockade	With blockade
HR						
preinjection (beats/min)	164 ± 15	153 ± 3	169 ± 11	214 ± 10	163 ± 7	230 ± 11*
maximum change (%Δ)	−72 ± 18	0 ± 4*	−55 ± 13	4 ± 3*	−64 ± 14	0 ± 0*
MAP						
preinjection (mm Hg)	67 ± 3	69 ± 3	65 ± 2	70 ± 2	75 ± 3	58 ± 2*
maximum change (%Δ)	−43 ± 10	0 ± 2*	−29 ± 5	−6 ± 5*	−49 ± 6	4 ± 8*
Q̄						
preinjection (kHz)	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.5 ± 0.2	1.9 ± 0.1	2.0 ± 0.2
maximum change (%Δ)	69 ± 10	6 ± 2*	55 ± 8	36 ± 16	70 ± 13	12 ± 7*
R						
preinjection (mm Hg/kHz)	34 ± 3	36 ± 2	33 ± 3	29 ± 3	41 ± 2	31 ± 4
maximum change (%Δ)	−46 ± 5	0 ± 3*	−40 ± 4	−30 ± 6	−55 ± 6	−18 ± 3*

[a] Opioid antagonism with naloxone hydrochloride (1 mg/kg i.v.) (*n* = 5).
[b] Muscarinic blockade with atropine methyl nitrate (0.1 mg/kg i.v.) (*n* = 7).
[c] Ganglionic blockade with chlorisondamine hydrochloride (2 mg/kg i.v.) (*n* = 5).
* Significantly different (P < .05) from that without blockade.

mENK

ARTERIAL
PRESSURE
(mmHg)
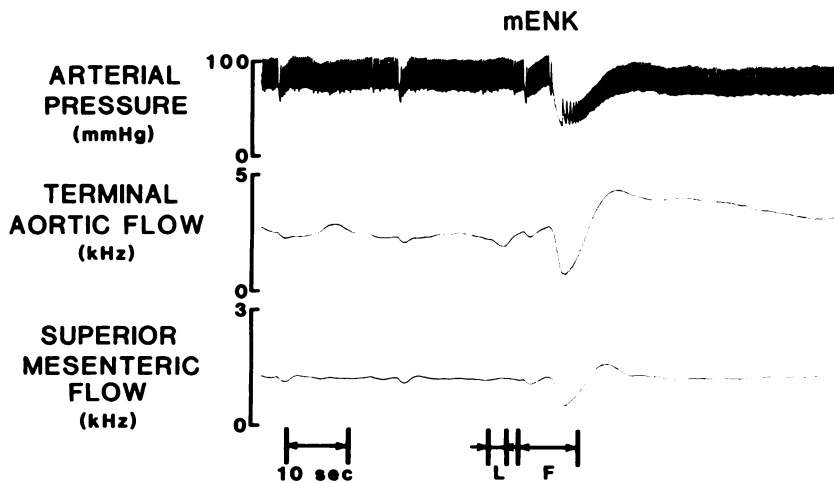
TERMINAL
AORTIC FLOW
(kHz)

SUPERIOR
MESENTERIC
FLOW
(kHz)

Fig. 5. Change in superior mesenteric and terminal aortic blood flow after injection of mENK (30 μg/kg) into the terminal aorta. Abbreviations are as in figure 2.

of conscious rabbits. Utilizing this model, we found that i.a. administration of mENK produced transient, dose-dependent decreases in mean aortic pressure and heart rate and a biphasic response in mean terminal aortic blood flow. The flow response was characterized by an initial, rapid decrease followed by a dose-dependent, secondary increase. The hemodynamic effects of mENK were mediated by opioid receptors because naloxone eliminated those effects.

The bradycardia after mENK was due to increased vagal activity to the heart. Peripheral muscarinic blockade with atropine methyl nitrate or ganglionic blockade with chlorisondamine abolished the decrease. Muscarinic blockade also abolished the initial decrease in hindquarter blood flow and significantly reduced the decrease in mean arterial pressure. This suggests that these events were secondary to the bradycardia.

The secondary increase in flow was due to a decrease in vascular resistance. Atropine did not alter this response. Thus, it was not simply a reactive hyperemic response to the decreased blood flow during the bradycardia. The decreased vascular resistance induced by mENK may have been mediated locally through a direct action on the vascular smooth muscle or by inhibition of the release of norepinephrine from the sympathetic nerve terminals (Illes et al., 1985). However, chlorisondamine significantly reduced the resistance decrease after i.a. mENK. This suggests that the vasodilation was probably the result of a decrease in sympathetic tone rather than a direct action of mENK on vascular smooth muscle.

It is possible that the vasodilatory effects of chlorisondamine pretreatment resulted in blood vessels being unable to dilate further in response to mENK or any other substance. We tested this possibility in two rabbits by injecting nitroglycerin (20 μg/kg i.a.), a known vasodilator, after treatment with chlorisondamine. Nitroglycerin more than doubled mean flow in the terminal aorta. Thus, chlorisondamine pretreatment did not limit our ability to detect decreases in vascular resistance. In addition, the simultaneous response in a proximal vascular bed does not support either direct effects of mENK or local actions on sympathetic nerve terminals. The hemodynamic response to mENK was mediated by increased parasympathetic activity to the heart and decreased sympathetic activity to the peripheral vasculature. The simultaneous change in resistance in two separate vascular beds supports the idea of a neural reflex.

It has been known for some time that i.v. morphine produces vagal bradycardia and hypotension (Evans et al., 1952; Fennessy and Rattray, 1971) due to stimulation of cardiopulmonary

vagal afferents. Activation of vagal afferents from the lungs by enkephalin-related peptides produces cardiovascular changes similar to those of morphine (Wei et al., 1980; Sapru et al., 1981). This may explain the i.v. response to mENK in the present study. However, the lungs were not the origin of the response to i.a. mENK. The more rapid onset after i.a. than after i.v. mENK indicated that the response originated in the hindquarters. If the peptide injected i.a. acted centrally or at a peripheral site other than the hindquarters, the onset of the response after i.v. mENK would have occurred at a shorter latency. Thus, our study is the first to demonstrate afferents for these opioid peptide-mediated depressor reflexes at sites other than in the lung.

The physiologic or pathophysiologic relevance of such reflexes remains to be demonstrated. A possible role for endogenous opioid peptides has been suggested in several experimental shock models. It is possible that these peptides, once released by the adrenal medulla and sympathetic nerve endings during shock, activate depressor reflexes limiting sympathetic activation and thus contribute to the hypotension. A broader distribution of these reflexes might enhance this function.

We have demonstrated a mechanism by which circulating mENK may act to decrease peripheral vascular resistance. When delivered directly to the hindquarters, mENK activates a reflex. The results include decreased vascular resistance in the target bed and in other vasculatures remote from the site of injection. The decrease in resistance was mediated by withdrawal of sympathetic tone. There appeared to be no direct effect of mENK on the vasculature.

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