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Effects of Methionine-Enkephalin on Intestinal Circulation and Oxygen Consumption[1] (40928)

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Abstract. The effects of intra-arterial administration of Met-enkephalin upon intestinal blood flow, oxygen consumption, intestinal motor activity, and distribution of blood flow to the compartments of the gut wall were measured in anesthetized dogs before and after blockade of opiate receptors with naloxone. Blood flow to a segment of distal ileum was measured with an electromagnetic blood flow meter and intestinal oxygen extraction was measured spectrophotometrically. Oxygen uptake was calculated as the product of oxygen extraction and total blood flow. Changes in blood flow distribution were estimated from the distribution of radiolabeled microspheres. Motor activity was monitored from changes in intraluminal pressure. In dogs prior to blockade of opiate receptors, Met-enkephalin induced a dose-related increase in mesenteric blood flow, oxygen extraction and consumption, and intestinal motor activity. A significant increase in blood flow to the muscularis was also observed. The intestinal vasodilator, metabolic, and motor responses to Met-enkephalin were abolished by blockade of opiate receptors with naloxone. The results of our study indicate that Met-enkephalin causes an increase in intestinal motor activity and an increase in the metabolic demand for oxygen. The primary effect probably results in smooth muscle relaxation in intestinal arterioles and precapillary sphincters, thereby increasing intestinal blood flow and oxygen consumption. We conclude that opiate receptors may be involved in the regulation of intestinal motor function.

Enkephalins are morphinomimetic pentapeptides with a wide spectrum of biological actions (1). Immunohistochemical localization of enkephalins has demonstrated their presence within the central nervous system (1–3), peripheral neurons (3, 4), and in the gastrointestinal tract, particularly in the mucosa of the gastric antrum and the upper small intestine (5, 6). These peptides also exhibit cardiovascular effects when administered centrally (7, 8) or peripherally (9, 10). We have previously reported that local administration of methionine-enkephalin (Met-enkephalin) consistently increases gastric mucosal and intestinal blood flow (11–13) and stimulates intestinal motor activity in a manner similar to that observed with morphine (12, 14).

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The present study was undertaken to investigate the effects of Met-enkephalin on intestinal circulation, oxygen consumption, and motor activity. Additionally, the intestinal vascular, metabolic, and motor responses to the peptide were tested after blockade of opiate receptors with naloxone.

Methods. A total of 12 mongrel dogs of either sex, weighing 18 to 27 kg, were used in this study. Animals were fasted for 24 hr before being anesthetized with intravenous sodium pentobarbital (30 mg/kg). A femoral vein was catheterized for injection of supplemental anesthesia and another catheter was inserted into a femoral artery for measurement of systemic arterial blood pressure with a strain gauge transducer (Model 1280, Hewlett-Packard Co., Palo Alto, Calif.). Following a midline laparotomy, a distal trunk of the superior mesenteric artery supplying a segment of distal ileum was exposed and a side branch of the artery was cannulated for infusion of drugs. The ends of the intestinal segment supplied by this trunk were ligated to block collateral circulation. The average weight of these seg-

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ments was 150 g. An electromagnetic blood flow transducer of appropriate internal diameter was positioned around the mesenteric artery and connected to a blood flow amplifier (SWF-4RD, Zepeda Instruments, Seattle, Wash.). Zero flow was obtained by momentarily occluding the artery downstream from the blood flow transducer. A side branch of the mesenteric vein draining this intestinal segment and a femoral artery were cannulated. Following heparin administration a constant flow pump (Holter, Model 911) circulated blood at 5.5 ml/min from these vessels through the arterial and venous cuvettes of a photometric arteriovenous oxygen content difference analyzer (A-Vox Systems, San Antonio, Tex.) (15). Arterial and venous effluents from the apparatus were then pumped back to the circulation via a femoral vein. Intestinal oxygen consumption ($\dot{V}O_2$) was calculated as the product of the arteriovenous oxygen difference (A-VO_2) and the total blood flow of the intestinal segment (MBF).

The intraluminal pressure of the intestinal segment was measured with a saline-filled, open tip polyvinyl tube inserted into the lumen and connected to a pressure transducer (Hewlett-Packard 1280C). The mean motility index (MMI) was calculated by dividing the sum of the heights of all contractions during a 10-min period by the number of contractions in the same time period (16). Arterial pressure, MBF, A-VO_2, and intraluminal pressure were monitored in all experiments on a direct-wiring recorder (Hewlett-Packard Model 7759A). A catheter was passed from the femoral artery and aorta into the left ventricle for injection of radiolabeled microspheres (3M, Minneapolis, Minn.), so that changes in blood flow distribution to the mucosa–submucosa and muscle layers of the gut segment could be determined as previously described (17). Just prior to injection, vials containing microspheres in 10% dextran were shaken and agitated on a vortex mixer for 10 min to ensure homogeneity. With each injection approximately 400,000 to 600,000 microspheres labeled with one isotope were introduced into the left ventricle. Injection of microspheres with different labels was made during the control period and during administration of the drugs. The mucosal and submucosal layers were then stripped from the muscular layer. The tissue layers were placed in preweighed plastic counting vials, the vials were weighed, and the radioactivity was determined in a gamma spectrometer (Packard Instruments). Fractional blood flow (FBF) to the muscularis was calculated as the product of MBF and the percentage of microsphere distribution to the muscularis.

Met-enkephalin (in saline) was infused intra-arterially for 10 min at each of the following doses: 0.03, 0.06, 0.125, 0.25, 0.5, and 1.0 μg/kg-min. The effects of opiate receptor blockade with naloxone (6 mg/kg-min) on the response to Met-enkephalin (0.5 μg/kg-min) were also determined. Naloxone dissolved in saline was administered over a 5-min period directly into the mesenteric artery in these experiments. The data from all experiments were evaluated statistically with Student's t-test for paired observations. Alterations from control were expressed as the percentage change. The significance of these changes was assumed at $P < 0.05$.

Results. After surgical preparation of the gut, time was allowed for MBF, A-VO_2, and pressure in the lumen to stabilize for 20 min. Control values obtained from 12 dogs were as follows: MBF, 63.0 ± 7.2 ml/min-100 g tissue; A-VO_2, 3.8 ± 0.4 ml O_2/100 ml blood; $\dot{V}O_2$, 2.4 ± 0.6 ml O_2/min-100 g; and MMI, 10.0 ± 1.0 mm Hg. Mean systemic arterial pressure ranged between 110 and 150 mm Hg and was not significantly changed from control during drug administration. Control intramural distribution of intestinal blood flow was 73.0 ± 6.0% for the mucosal–submucosal compartment and 27.0 ± 6.0% for the muscularis. Control FBF was 17.0 ± 4.2 ml/min-100 g tissue.

During intra-arterial administration of Met-enkephalin in doses of 0.03, 0.06, 0.125, 0.25, 0.5, and 1.0 μg/kg-min, dose-dependent increases in MBF, A-VO_2, $\dot{V}O_2$, and MMI were observed (Fig. 1). All parameters reached a peak value at a dose of 0.5 μg/kg-min when MBF was increased 22.8 ± 4.2% ($P < 0.001$), A-VO_2 increased 33.1 ± 3.6% ($P < 0.001$), $\dot{V}O_2$ increased 52.8 ± 6.7% ($P < 0.001$), and MMI in-

creased 107.0 ± 11.2% ($P < 0.001$) (Fig. 1). A representative tracing illustrating these changes at 0.5 μg/kg-min Met-enkephalin is shown in Fig. 2. Microsphere distribution was examined only at the end of infusion of this dose and showed a significant increase in FBF of 49.6 ± 7.2% ($P < 0.001$) (Fig. 3). Naloxone administration alone failed to affect any circulatory parameter significangly but caused a 32.0 ± 4.2% ($P < 0.005$) decrease in MMI (Fig. 3). Infusion of Met-enkephalin after blockade of opiate receptors with naloxone failed to affect any measured parameter significantly.

Discussion. In the present study we found that Met-enkephalin is a vasodilator of the canine intestinal circulation. Furthermore, there is a direct relationship between the dose of the drug administered intra-arterially and the subsequent increase in flow. The Met-enkephalin-induced increase in intestinal blood flow observed in the current study corresponds with previous reports showing that Met-enkephalin, when administered locally, is a vasodilator substance under both *in vivo* (9) and *in vitro* (10) conditions. However, it should be noted that in a few experiments (not included in this report), infusion of Met-enkephalin at low doses produced a small decrease in mesenteric blood flow. Since systemic arterial pressure was unaffected by infusions of the drug into the superior mesenteric artery, Met-enkephalin appears to be a local vasodilator of the intestinal circulation.

We also found that Met-enkephalin evoked significant increases in intestinal

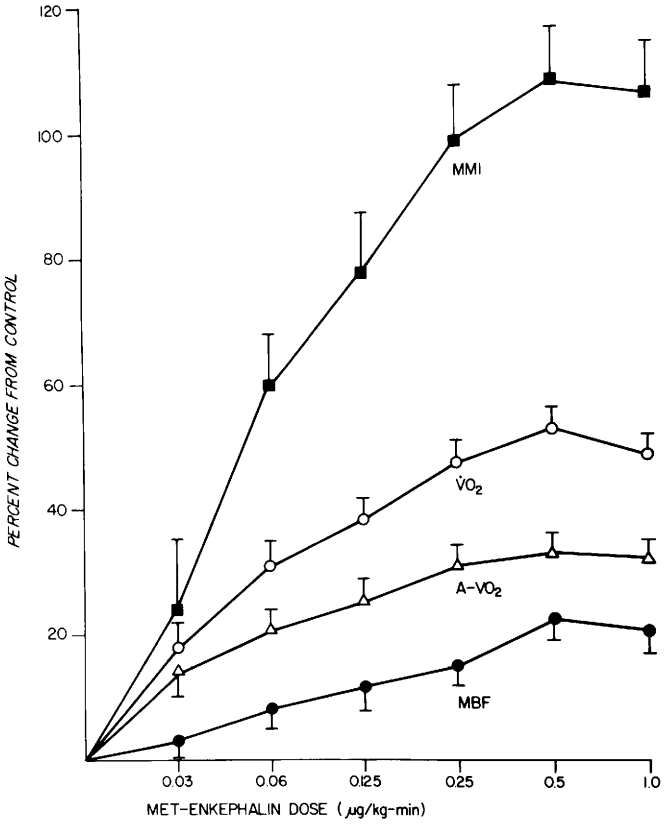

FIG. 1. Effects of intra-arterial infusions of Met-enkephalin on mesenteric blood flow (MBF), arteriovenous oxygen content difference (A-VO_2), intestinal oxygen consumption ($\dot{V}O_2$), and mean motility index (MMI). Mean values ± SEM are presented for seven dogs. All values shown except for MBF at a dose of 0.03 μg/kg-min are significantly increased from control.

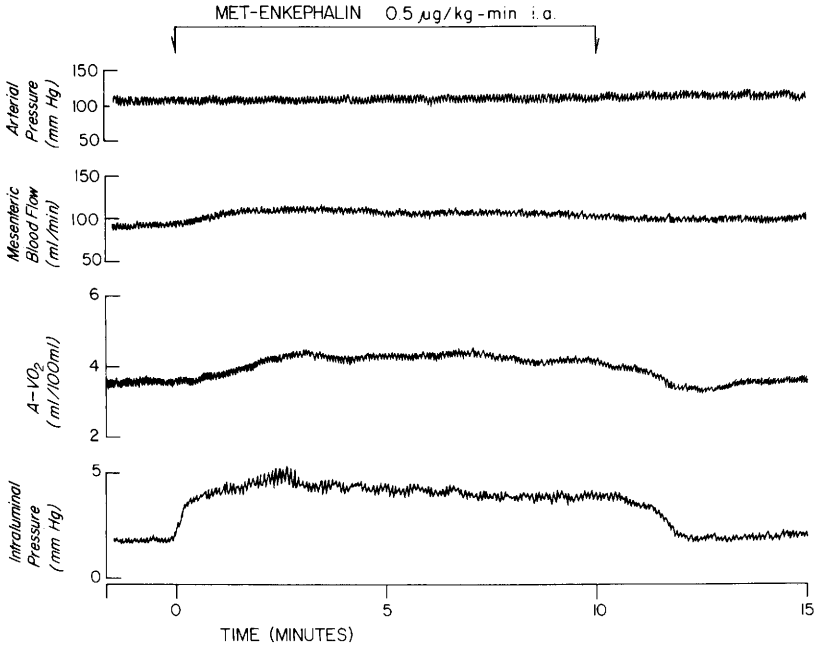

FIG. 2. A representative recording from one experiment showing the effects of direct intra-arterial infusion of Met-enkephalin (0.5 μg/kg-min) on systemic arterial pressure, mesenteric blood flow, arteriovenous oxygen content difference (A-VO_2), and intraluminal pressure.

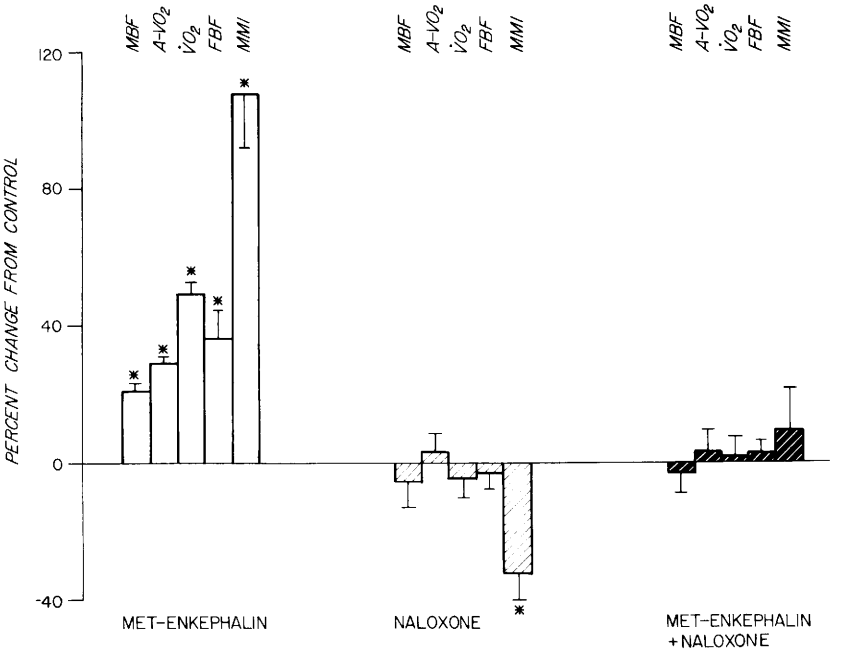

FIG. 3. Effects of intra-arterial infusion of Met-enkephalin (0.5 μg/kg-min) on MBF, A-VO_2, $\dot{V}O_2$, FBF, and MMI before and after naloxone. Mean ± SEM for seven dogs. Asterisks indicate significant difference from control to at least the $P < 0.05$ level.

oxygen extraction and uptake, and increases in the distribution of blood flow to the muscular compartment of the gut wall. The effects of Met-enkephalin were associated with an increase in intestinal motility. Similar findings with intestinal motility were previously reported with morphine (14). Presumably Met-enkephalin exerts its influence upon intestinal blood flow by diminishing the vasoconstrictor tone of the arteriolar smooth muscle, which regulates resistance to the total flow of blood through the gut, and the smooth muscle of the precapillary sphincters, which regulate the flow of blood through the capillaries (18).

The increase in intestinal oxygen extraction caused by Met-enkephalin is probably secondary to an increased metabolism of the intestinal musculature. However, since oxygen consumption is a product of blood flow and oxygen extraction, the increase in oxygen consumption produced by Met-enkephalin could have resulted from stimulation of intestinal metabolism with increased demand for oxygen and from an increase in blood flow.

Met-enkephalin had a marked excitatory effect on intestinal motility. These changes could have affected intestinal circulation secondary to the modifications of intestinal intramural pressure. Therefore, the observed mesenteric vasodilation and increased oxygen consumption could have been the consequence of increased metabolic demand for oxygen or accumulation and release of local dilator factors produced by increased metabolic activity in the gut (19). These possibilities are supported by our experiments with naloxone, in which blood flow and oxygen consumption were significantly different during Met-enkephalin infusion before and after naloxone, that is, with and without motility changes.

Numerous reports have suggested that Met-enkephalin exerts its physiological action through release of serotonin (20) or histamine (21). However, we have previously reported that histamine-induced intestinal vasodilation was accompanied by a decrease in intestinal oxygen extraction (22) which suggests that this agent is not involved in intestinal vascular and metabolic responses to Met-enkephalin.

The specific opiate receptor for the central and peripheral neural effects of Met-enkephalin is blocked by naloxone (23). In the current study the intestinal vasodilator, motor and metabolic responses to Met-enkephalin were also abolished by naloxone, a finding which suggests that opiate receptors may be involved in the mechanism of action of Met-enkephalin on both intestinal visceral and vascular smooth muscle effectors of the gut. Since blockade of the opiate receptor in the resting gut did not change blood flow but significantly decreased motility, it may be that opiate receptors are involved in the regulation of intestinal motor activity but not indirect control of the circulation.

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