Naloxone Improves Splanchnic Perfusion in Conscious Dogs through Effects on the Central Nervous System

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Background: In patients undergoing colonoscopy, naloxone has vasodilative properties. However, it remains unclear whether this effect is mediated by central or peripheral mechanisms. The aim of this study was to investigate whether these effects are mediated by an effect of naloxone on the central nervous system.

Methods: Twenty dogs were chronically instrumented for measurement of hemodynamic parameters. Splanchnic blood flow was determined using colored microspheres. Transthoracic echocardiographic examinations were performed to measure cardiac output. In each animal, two experiments were performed in a random order: experiment 1 was determination of splanchnic blood flow before and 5 min after intravenous administration of naloxone (63 μg/kg), and experiment 2 was determination of splanchnic blood flow before and 5 min after administration of naloxone methiodide (63 μg/kg), which does not cross the blood–brain barrier.

Results: Naloxone, but not naloxone methiodide, significantly increased blood flow to the stomach (from 0.41 ± 0.022 to 0.9 ± 0.016# ml · g^{-1} · min^{-1} with naloxone), jejunum (from 0.31 ± 0.024 to 0.83 ± 0.083# ml · g^{-1} · min^{-1} with naloxone), colon (from 0.41 ± 0.057 to 0.68 ± 0.008# ml · g^{-1} · min^{-1} with naloxone), spleen (from 1.45 ± 0.21 to 2.13 ± 0.25# ml · g^{-1} · min^{-1} with naloxone), pancreas (from 0.97 ± 0.021 to 1.25 ± 0.005# ml · g^{-1} · min^{-1} with naloxone), and kidneys (from 3.24 ± 0.108 to 5.31 ± 0.26# ml · g^{-1} · min^{-1} with naloxone), without altering cardiac output or arterial blood pressure in conscious dogs. There were no differences in the hemodynamics or cardiac output between the two experiments. Data are presented as mean ± SD.

Conclusions: The increased splanchnic perfusion after naloxone is not caused by direct peripheral vascular effects or increased cardiac output. Indirect vasodilative effects on splanchnic vessels mediated by actions of naloxone on the central nervous system account for the increased gastrointestinal perfusion after naloxone in dogs.

IN patients undergoing colonoscopy, naloxone—a nonspecific opioid receptor antagonist—has vasodilative properties. However, conclusive hemodynamic parameters and cardiac output effects associated with the drug have not yet been established.[1] It is therefore still unclear whether these effects are caused by an intrinsic vasodilative effect of naloxone or a positive inotropic one.[2] It is also still unclear at present whether the effects concerned are mediated centrally or are caused by a peripheral mechanism.

To clarify these questions, the current investigation was conducted to examine the effects of naloxone and naloxone methiodide, which does not cross the blood–brain barrier, on splanchnic perfusion. Simultaneous hemodynamic and transthoracic echocardiographic examinations were performed to distinguish between the vasodilative and hemodynamic effects of naloxone and naloxone methiodide. To avoid the release of endogenous opioid peptides (EOPs), the experiments were conducted in a chronically instrumented experimental model.[3]

Materials and Methods

The experimental protocol was in accordance with the American Physiologic Society's "Guiding Principles for the Care and Use of Animals." It was approved by the District Government of Münster. After overnight fasting, 20 mongrel dogs (either sex; weight, 24–29 kg) received intramuscular premedication with 1 mg/kg piritramide and 5 mg/kg ketamine. The animals were anesthetized intravenously with 5 mg/kg propofol. After tracheal intubation, anesthesia was maintained with isoflurane in a mixture of oxygen in air (35% oxygen). Perioperative antibiotic prophylaxis was achieved with 30 mg/kg cefamandole. Details of the instrumentation methods have been published previously.[4] Briefly, a left thoracotomy was performed in the fifth intercostal space during aseptic conditions. Eighteen-gauge catheters were inserted into the descending aorta and the left atrium for pressure measurement, injection of microspheres, injection of naloxone or naloxone methiodide, and withdrawal of blood. After closure of the thorax, all leads were tunneled subcutaneously and exteriorized between the scapulae. Postoperative analgesia was performed with piritramide. After instrumentation, the animals were trained daily to accustom them to the experimental environment and to ensure that they were able to lie quietly in the cage when connected to the data acquisition system. Aortic and left atrial pressures were measured using disposable pressure transducers. Pressure signals were processed using a six-channel pulsed Doppler system (Baylor College of Medicine, Houston, TX). All signals were digitally recorded. Experiments were only conducted after the animals had recovered completely

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# Indicates significance.

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Table 1. MAP, LAP, HR, and CO of the Awake Animals before, during, and after Application of Naloxone or Naloxone Methiodide at Predetermined Time Points

		MI at BL	NI or NMI	MI		
	Time (min)		0	5	15	30
MAP (mmHg)	Naloxone	113 ± 11	114 ± 7	118 ± 8	113 ± 4	112 ± 6
	Naloxone methiodide	115 ± 9	117 ± 10	116 ± 8	114 ± 4	118 ± 6
LAP (mmHg)	Naloxone	2.1 ± 1.9	1.8 ± 1.4	1.9 ± 2.2	2.3 ± 1.3	2.2 ± 1.7
	Naloxone methiodide	2.9 ± 1.5	2.2 ± 1.9	2.5 ± 1.5	2.3 ± 1.8	2.1 ± 2.6
HR (bpm)	Naloxone	92 ± 7	93 ± 5	94 ± 6	91 ± 8	95 ± 4
	Naloxone methiodide	89 ± 6	94 ± 9	92 ± 5	91 ± 4	93 ± 8
CO (L/min)	Naloxone	2.3 ± 0.2	2.2 ± 0.3	2.0 ± 0.2	2.4 ± 0.3	2.2 ± 0.2
	Naloxone methiodide	2.4 ± 0.3	2.3 ± 0.2	2.2 ± 0.3	2.4 ± 0.2	2.3 ± 0.2

Data are presented as mean values ± SD.

MAP = mean arterial pressure; LAP = left atrial pressure; HR = heart rate; CO = cardiac output; BL = baseline; MI = microsphere injection; NI = naloxone injection; NMI = naloxone methiodide injection.

(at least 12 days after surgery) from the instrumentation and when measurements of blood gas values and hemodynamic variables showed normal values.

Splanchnic blood flow was measured using colored microspheres (Triton Technology, San Diego, CA). For each measurement, a total of 9×10^6 microspheres suspended in a volume of 3 ml were injected into the left atrium. The reference blood sample was withdrawn from the aortic catheter at a rate of 10 ml/min. After the animals had been killed, the abdominal organs (stomach, jejunum, colon, spleen, liver, pancreas, and kidney) were dissected, and tissue samples were obtained. Measurement of microspheres in the tissue samples was performed as described previously.[5]

The experimental design was as follows. All 20 dogs were randomly assigned to one of two groups (group 1 with naloxone and group 2 with naloxone methiodide; 10 dogs in each group). All of the animals in each group were exposed to the following two experimental conditions. In experimental condition 1, splanchnic blood flow was measured at baseline in the awake state. In experimental condition 2, immediately after the baseline measurement, a bolus of 63 μg/kg naloxone (Curamed Pharma Ltd. Gmbh, Karlsruhe, Germany) or naloxone methiodide (Sigma-Aldrich Chemie Ltd., Deisenhofen, Germany) was administered through the left atrial catheter. This dose was chosen on the basis of previously published results in dogs.[3] Five minutes after the bolus application, splanchnic blood was measured again.

Transthoracic echocardiographic examinations were conducted to determine cardiac output at the following time points: baseline, immediately after bolus administration of the study drug, and 5, 15, and 30 min after bolus administration.

The experiments were conducted in chronically instrumented conscious dogs to avoid the effects of acute surgical trauma, anesthesia, volume and ion imbalances, and temperature on the experiments.

Echocardiography

Transthoracic echocardiographic examinations to measure cardiac output were conducted using the standard apical four-chamber view (2–4 MHz Fusion Imaging Linear Scanner, Sonos 5500; Hewlett-Packard Medical Products, Andover, MA). The sonic frequency used for echocardiography was 1.8 MHz (second harmonic imaging, with a transmission frequency of 1.8 MHz and a receiving frequency of 3.6 MHz). All examinations were conducted by the same individual (Jörg Stypmann, M.D., Fellow of the German Cardiac Society, UKM–Medizinische Klinik und Poliklinik C, Kardiologie und Angiologie, Münster, Germany), recorded on videotape, and analyzed after the experiments with the observer blinded with respect to the experimental condition. An echocardiographic assessment was conducted at baseline and after application of naloxone or naloxone methiodide.

Statistical Analysis

The data were analyzed using repeated-measures two-way analysis of variance followed by Bonferroni-corrected Student t test, whenever appropriate. $P < 0.05$ was regarded as significant. Data are presented as mean ± SD.

Results

Mean Arterial Blood Pressure, Heart Rate, Left Atrial Pressure, and Cardiac Output

There were no significant changes in mean arterial pressure, heart rate, left atrial pressure, or cardiac output, with or without naloxone or naloxone methiodide (table 1).

Splanchnic Blood Flow

Naloxone, but not naloxone methiodide, led to a significant increase in blood flow to the stomach, jejunum, colon, spleen, pancreas, and kidney (table 2). Measure-

Table 2. Blood Flow (ml · g^{-1} · min^{-1}) to Splanchnic Organs in Awake Dogs with Naloxone and with Naloxone Methiodide

	Baseline	Naloxone	Baseline	Naloxone Methiodide
Stomach	0.41 ± 0.022	0.9 ± 0.016*	0.39 ± 0.084	0.42 ± 0.079
Jejunum	0.31 ± 0.024	0.83 ± 0.083*	0.34 ± 0.016	0.33 ± 0.12
Colon	0.41 ± 0.057	0.68 ± 0.008*	0.37 ± 0.054	0.38 ± 0.048
Spleen	1.45 ± 0.21	2.13 ± 0.25*	1.44 ± 0.1	1.48 ± 0.54
Liver	0.12 ± 0.026	0.11 ± 0.032	0.15 ± 0.089	0.13 ± 0.021
Pancreas	0.97 ± 0.021	1.25 ± 0.005*	0.74 ± 0.016	0.83 ± 0.16
Kidney	3.24 ± 0.108	5.31 ± 0.26*	3.85 ± 0.045	3.31 ± 0.34

Data are presented as mean values ± SD.

* Significant in comparison with the control baseline.

ments were made 5 min after the injection of naloxone or naloxone methiodide.

Discussion

In chronically instrumented dogs, naloxone improved splanchnic blood flow in all organs with the exception of the liver, without changing hemodynamic parameters. Naloxone methiodide, which does not cross the blood–brain barrier, did not induce any changes in splanchnic perfusion or hemodynamic parameters.

Naloxone is generally considered to be a pure μ-receptor antagonist.[6] However, naloxone may also cause several μ-receptor–independent intrinsic effects on the gastrointestinal tract, such as inhibition of gastric emptying in the rat,[7] dose-dependent vasoactivity on splanchnic blood vessels,[8] or positive inotropic effects on the cardiac muscle, both *in vitro* and *in vivo*.[9] The ability of naloxone to enhance the colonoscopic appearance of normal colon vasculature and colon vascular ectasias, described by Brandt and Spinnell[1] in humans undergoing colonoscopy, may thus represent intrinsic vasodilative effects on splanchnic vessels or positive inotropic effects on the myocardium. In the study by Brandt and Spinnell,[1] hemodynamic parameters were not measured. Therefore, a naloxone-induced positive inotropic effect, which increases splanchnic perfusion, cannot be excluded. To clarify this question, an intact autonomic nervous system is necessary.

In the current study, only naloxone—which may have peripheral and central nervous system effects, as it penetrates the blood–brain barrier—improved splanchnic blood flow without inducing hemodynamic changes. The failure of naloxone methiodide—which only acts peripherally[10,11] to affect splanchnic blood flow[12]—to produce peripheral and central nervous system effects in this study proves that this effect of naloxone on splanchnic perfusion is caused by centrally mediated agonistic vasodilative effects.

Naloxone methiodide is the quaternary salt of naloxone that, like the parent compound, is a nonselective antagonist at opiate receptors. Naloxone methiodide was chosen because it is soluble in water and does not

cross the blood–brain barrier. One possible explanation for the failure of naloxone methiodide to influence splanchnic blood flow might be a poor interaction of this drug with opioid receptors in the periphery. However, several investigators have clearly shown the peripheral antagonistic effects of naloxone methiodide on opiate receptors, when administered in peripheral veins and subcutaneously, in several experimental studies.[10,11,13–15] In addition, antagonistic effects, especially on splanchnic opiate receptors, mediated by quaternary naloxone analogs, have been reliably demonstrated by, *e.g.*, Jang *et al.*,[16] Romanovsky *et al.*,[17] and Sharma *et al.*[12] EOPs are found in central areas near the cardiovascular centers of the brain, in the hypothalamus, and peripherally in the myocardium.[18] The location of EOPs in the nucleus of the solitary tract suggests central involvement of EOPs in the modulation of gastrointestinal functions.[19] Peripheral opioid receptors have been identified on the enteric neurons,[20] enterocytes,[21] and membrane fractions obtained from pyloric smooth muscle.[22] In chronically instrumented dogs with no physiologic disturbances such as myocardial ischemia or sepsis, EOPs are not present.[3,23] Antagonistic effects of naloxone in this investigation were therefore unlikely. Because splanchnic blood flow increased without significant positive inotropic effects, we hypothesize that this effect of naloxone is caused by agonistic vasodilative properties.

A limitation of this work is that the blood flows to extrasplanchnic vascular beds were not examined; therefore, final statements about blood flows to extraintestinal organs are not possible. During physiologic situations, a substantial increase in blood flow to the splanchnic and renal beds without a decrease in peripheral blood pressure, as shown in our study, is followed by an increase in cardiac output. However, with the absence of an increased cardiac output and decrease in mean arterial blood pressure, the only plausible explanation for the observed effects is a redistribution of blood flow to muscle, fat, or skin, because cardiac output did not increase. In addition, consistent with this phenomenon, vascular shunts are generating, at least in early shock stages, without macrohemodynamic signs.[24,25] However, measurements of splanchnic blood with microspheres permits only a snapshot at a deter-

mined point of time. The first measurement after administration of naloxone or naloxone methiodide was performed 5 min after the bolus injection. It may therefore have been possible that hemodynamic changes, *e.g.*, a reactive increase in cardiac output, occurred in the interval between the microsphere measurements. If a continuous determination of blood flow was possible in this model, the results might look different. Therefore, it is not possible to describe the effects of naloxone over time. Maybe only a continuous infusion of naloxone and therefore a permanent blockade of opiate receptors leads to a reactive increase in cardiac output. In general, naloxone and naloxone methiodide act on opiate receptors only for a few minutes. The duration of action is therefore possibly too short to show the expected increase in cardiac output. Another explanation is the relatively low dosage we used in our experiments: 63 μg/kg of naloxone or naloxone methiodide is, referring to other animal studies,[26–28] a small dosage. Therefore, the probability for a cumulation of naloxone or naloxone methiodide is much lower according to most other studies. These two facts might explain, at least in part, the absent cardiac output increase.

It is concluded from the findings presented here that, in chronically instrumented dogs, naloxone improves splanchnic perfusion *per se* through a centrally mediated mechanism. Central effects of naloxone are predominantly located in the hypothalamus.[29] Identification of the central receptors mediating this beneficial effect may lead to the development of specific ligands devoid of the undesired μ-receptor antagonism, thus offering a treatment option for improving splanchnic perfusion, *e.g.*, in critically ill patients.

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