Role of Endogenous Opioids and Central Opioid Receptors in Cerebral Cortical Blood Flow Autoregulation

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Abstract To examine the role of endogenous opioids in autoregulatory maintenance of cerebral cortical blood flow (CoBF), CoBF was measured continuously by laser-Doppler flowmetry during changes in arterial pressure. Experiments were conducted on pentobarbital sodium-anesthetized adult mongrel dogs. Mean arterial pressure (MAP) was decreased or increased by inflating a perivascular occluder placed around the inferior vena cava or the thoracic descending aorta, respectively. To exclude the influence of the baroreceptor reflex on the autoregulatory maintenance of CoBF, all experiments were conducted on dogs with bilateral carotid sinus denervation plus vagotomy. CoBF was well maintained within its normal range despite large changes in MAP. Intravenous injection of naloxone (2.5 μ mol/kg), an opioid receptor antagonist, significantly impaired the autoregulatory maintenance of CoBF during the decrease in MAP. On the other hand, intravenous injection of methyl naloxone (2.5 μ mol/kg), which does not cross the blood-brain barrier, did not exert any significant effect on the MAP-CoBF relationship. Furthermore, intracerebroventricular injection of a smaller dose of naloxone (2.5 nmol/kg) significantly impaired the autoregulatory maintenance of CoBF during the decrease in MAP, as the larger dose of intravenous naloxone (2.5 μ mol/kg) did. On the other hand, intravenous injection of the smaller dose of naloxone did not exert any significant effect on the MAP-CoBF relationship. These findings suggest that endogenous opioids and central opioid receptors may be partly involved in the CoBF autoregulatory mechanism. The endogenous opioids may modulate the autoregulatory vasodilation of the cerebral cortex during the decrease in MAP.

Key words: cerebral cortical blood flow, autoregulation, endogenous opioid, naloxone, laser-Doppler flowmetry.

In 1959, Lassen introduced the concept of cerebral blood flow (CBF) autoregulation [1]. CBF autoregulation maintains a constant blood flow to the brain by actively dilating resistance vessels as arterial blood pressure falls and by actively constricting the vessels as the pressure rises. Myogenic mechanism in vessels [2, 3], metabolic factors [4, 5], nervous system [6, 7], and cerebrovascular endothelial cells [8–11] are the candidate mechanisms of the autoregulation. Furthermore, it was suggested that endogenous opioids participated in the autoregulatory control of CBF [12, 13]. However, the role of endogenous opioids in CBF autoregulation has been reported by employing discontinuous measurement of CBF. In fact, in previous studies, CBF was measured by employing a microsphere technique [12] or a hydrogen clearance method [13]. Laser-Doppler flowmetry allows continuous monitoring of rapid changes in CBF and assessment of CBF autoregulation on a real time basis. Although the method can not measure absolute values for regional CBF, it is useful for measuring relative changes in the regional CBF [14]. Moreover, there is a good correlation between values obtained by laser-Doppler flowmetry and values obtained by the hydrogen clearance [14] or microsphere methods [15]. In the present study, therefore, we examined the role of endogenous opioids in the autoregulatory maintenance of cerebral cortical blood flow (CoBF) during changes in mean arterial pressure (MAP) by employing laser-Doppler flowmetry.

MATERIALS AND METHODS

Surgical preparation. Experiments were conducted on adult mongrel dogs ($n = 30$) weighing 8–12 kg anesthetized with pentobarbital sodium (25 mg/kg, i.v.). The animals were artificially ventilated (model SN-480-4, Shinano, Tokyo) through a tracheal cannula. To exclude the influence of the baroreceptor reflex on the autoregulatory maintenance of CoBF, all experiments were conducted on dogs with bilateral carotid sinus denervation plus vagotomy. Arterial pressure (AP) was monitored by a catheter inserted into the aortic arch via the femoral artery. A venous catheter was placed in the inferior vena cava via the femoral vein for administration of drugs. Left thoracotomy was performed through the fifth intercostal space and perivascular occluders were implanted around the thoracic descending aorta and the inferior vena cava. Then the chest was closed. The dog was turned prone and its head was fixed on the stereotaxic frame (Universal Stereotaxic Instrument, Summit Medical Co., Tokyo). A middle skin incision on the head was performed and a burr hole of 1 mm diameter was made on the left parietal bone at a site approximately 1.5 cm lateral to the midline and 2 cm posterior to the bregma. Then, a pin point hole was made in the dura mater without damaging the cerebral cortex. A plastic fiber attached to the tip of laser-Doppler flow probe (Type N, tip diameter of 0.3 mm, Advance, Tokyo) was inserted into the cerebral cortex through the hole and placed 5 mm below the cortical surface. In the preliminary experiment, we had confirmed that normal responsiveness to hypercap-

Fig. 1. Responses of arterial pressure (AP), mean arterial pressure (MAP), and cerebral cortical blood flow (CoBF) to inhalation of 5% CO₂ gas. CoBF was sensitively increased by inhalation of 5% CO₂ gas and returned to the control level when inhalation was stopped.

nia was maintained in 4 dogs. It was found that CoBF was sensitively increased by $69 \pm 7\%$ by inhalation of 5% CO₂ gas (Fig. 1). The other laser-Doppler flow probe (Type S, Advance, Tokyo) was placed on the right temporal muscle for measuring muscle blood flow (MBF). AP was measured using a Statham P23ID transducer which was connected to the previously inserted arterial catheter. The laser-Doppler flowmeters (Advance laser flowmeter, ALF 21, Advance, Tokyo) were then connected to flow probes for continuous monitoring of the CoBF and MBF. Electronic R-C Filters with a 2-s time constant were used to derive mean values of AP, CoBF and MBF.

Protocol. After stable values of MAP, MBF, and CoBF were obtained, experimental protocol was started. The MAP was slowly altered in a rampwise manner (0.25 mmHg/s) by inflation of the aortic occluder (Fig. 2) or the inferior vena caval occluder (Fig. 3). Approximately 4 min were allowed to elapse to alter

Fig. 2. Typical recordings of arterial pressure (AP), mean arterial pressure (MAP), cerebral cortical blood flow (CoBF), and muscle blood flow (MBF) during the increase in arterial pressure. MAP was increased in a rampwise manner ($+60$ mmHg) by inflation of the thoracic descending aortic occluder.

MAP (± 60 mmHg), then the occluder was deflated slowly. The order of changes in MAP, i.e., the increase or decrease in MAP, was randomized. Since it took approximately 3 min for recovery of pre-inflation levels of MAP, CoBF, and MBF, at least 5 min were allowed to elapse before the next experiment. The experimental runs with a decrease in MAP and an increase in MAP were performed twice, and average values obtained from the two experimental runs were pooled. In four dogs, arterial blood gas was measured before and during the occluder inflation. No significant influence was found on P_{aO_2} or P_{aCO_2} (Table 1). To examine the role of endogenous opioids in CoBF autoregulation, MAP-MBF and MAP-CoBF relationships were measured before and after an intravenous injection of opioid receptor antagonist, naloxone (naloxone hydrochloride, Sigma; $2.5 \mu\text{mol/kg}$, $n=9$). Furthermore, to examine whether naloxone acted on the peripheral side or the central side, the following two protocols were performed. First, methyl naloxone (MRZ 2593 BR, naloxone methobromide, Boehringer Ingelheim; $2.5 \mu\text{mol/kg}$, $n=9$), which does not cross the blood-brain barrier [16, 17], was injected instead of naloxone. The MAP-MBF and MAP-CoBF relationships were obtained before and after the intravenous injection of methyl naloxone. Second, a smaller dose of naloxone (2.5 nmol/kg in $10 \mu\text{l/kg}$ volume of saline) was injected either intravenously ($n=6$) or intracerebroventricularly (by using a 23-gauge, angiocath-

Fig. 3. Typical recordings of arterial pressure (AP), mean arterial pressure (MAP), cerebral cortical blood flow (CoBF), and muscle blood flow (MBF) during the decrease in arterial pressure. MAP was decreased in a rampwise manner (-60 mmHg) by inflation of the inferior vena caval occluder.

Table 1. Influence of changes in MAP on Pa_{O_2} and Pa_{CO_2} (mean $\pm$ SEM)

	Control	Changes in MAP ($+60$ mmHg)	Changes in MAP (-60 mmHg)
Pa_{O_2} (mmHg)	90.8 ± 2.2	91.2 ± 2.6	96.7 ± 1.3
Pa_{CO_2} (mmHg)	28.0 ± 1.1	26.3 ± 0.5	25.0 ± 0.6

$n = 4$.

eter, inserted into the left lateral cerebral ventricle; $n = 6$). The MAP-MBF and MAP-CoBF relationships were measured before and after the injection of the smaller dose of naloxone.

Statistical analysis. Results are expressed as the mean $\pm$ SEM. $p < 0.05$ was required for comparisons to be significant. The effects of intravenous or intracerebroventricular injection of naloxone and intravenous injection of methyl naloxone on the CoBF and MBF were analyzed by two-way analysis of variance for repeated measurements. When the F ratio exceeded the critical value, the method of Scheffe was applied to determine the significance of the differences between the data.

RESULTS

Typical recordings of AP, MAP, CoBF, and MBF are shown in Figs. 2 and 3. MBF decreased with decreasing MAP and increased with increasing MAP (Fig. 4). On the contrary, the CoBF was well maintained during changes in MAP. That is, when MAP was decreased by 60 mmHg, MBF and CoBF decreased by 69 ± 5 and $27 \pm 6\%$, respectively. When MAP was increased by 60 mmHg, MBF and CoBF increased by 85 ± 13 and $13 \pm 3\%$, respectively.

The baseline MAP was not significantly altered by intravenous injection of naloxone from 120.9 ± 3.6 to 118.5 ± 3.8 mmHg. Responses of MBF to the changes in MAP were not altered by the intravenous injection of naloxone ($2.5 \mu\text{mol/kg}$; Fig. 4). On the contrary, CoBF began to parallel the changes in MAP. In particular, the flow changes were significantly greater during the decrease in MAP. Before and after the intravenous injection of naloxone, CoBF was increased by 13 ± 3 and $18 \pm 8\%$, respectively, in response to the increase in MAP by 60 mmHg.

Fig. 4. The effect of intravenous injection of naloxone, an opioid receptor antagonist, on CoBF autoregulation ($n=9$): ΔMAP shows changes in MAP. ΔBF shows % changes in blood flow. Open circles and triangles indicate responses of CoBF and MBF before the injection of naloxone, respectively. Closed circles and triangles indicate responses of CoBF and MBF after the injection of naloxone, respectively. Values are mean $\pm$ SEM. * $p < 0.05$, compared with values before the injection of naloxone.

Before and after the intravenous injection of naloxone, CoBF decreased by 27 ± 6 and $42 \pm 5\%$, respectively, in response to the decrease in MAP by 60 mmHg.

To examine whether the site of action of naloxone was central or peripheral, methyl naloxone ($2.5 \mu\text{mol/kg}$), which does not cross the blood-brain barrier, was injected intravenously instead of naloxone. The baseline MAP was not significantly altered by the intravenous injection of methyl naloxone from 107.9 ± 3.6 to 109.6 ± 4.2 mmHg. The MAP-CoBF relationship was not altered by the injection (Fig. 5). This result suggests that naloxone acts on the central side. To further confirm the central action of naloxone, a smaller dose of naloxone (2.5 nmol/kg in $10 \mu\text{l/kg}$ volume of saline) was injected either intracerebroventricularly or intravenously. The MAP was not significantly altered by the smaller dose of intracerebroventricular injection of naloxone from 118.3 ± 4.3 to 126.2 ± 3.4 mmHg. The MAP-MBF relationship was not altered by the injection (Fig. 6). On the contrary, CoBF began to parallel the changes in MAP. In particular, the flow changes were significantly greater during the decrease in MAP. Before and after the intracerebroventricular injection of naloxone, CoBF was increased by 19 ± 4 and $33 \pm 5\%$, respectively, in response to the increase in MAP by 60 mmHg. Before and after the intracerebro-

Fig. 5. The effect of intravenous injection of methyl naloxone, an opioid receptor antagonist, on CoBF autoregulation ($n=9$): ΔMAP shows changes in MAP. ΔBF shows % changes in blood flow. Open circles and triangles indicate responses of CoBF and MBF before the injection of methyl naloxone, respectively. Closed circles and triangles indicate responses of CoBF and MBF after the injection of methyl naloxone, respectively. Values are mean $\pm$ SEM.

Fig. 6. The effect of intracerebroventricular injection of the smaller dose of naloxone (2.5 nmol/kg), an opioid receptor antagonist, on CoBF autoregulation ($n=6$): Δ MAP shows changes in MAP. Δ BF shows % changes in blood flow. Open circles and triangles indicate responses of CoBF and MBF before the injection of naloxone, respectively. Closed circles and triangles indicate responses of CoBF and MBF after the injection of naloxone, respectively. Values are mean $\pm$ SEM. * $p < 0.05$, compared with values before the injection of naloxone.

ventricular injection of naloxone, CoBF decreased by 24 ± 5 and $45 \pm 4\%$, respectively, in response to the decrease in MAP by 60 mmHg. The MAP was not significantly altered by intravenous injection of the smaller dose of naloxone (2.5 nmol/kg) from 119.4 ± 4.4 to 120.8 ± 4.1 mmHg. The intravenous injection of the smaller dose of naloxone had no effects on the MAP-CoBF and MAP-MBF relationship (Fig. 7).

DISCUSSION

Barzo *et al.* [18] demonstrated that the function of CBF autoregulation was strongly influenced by the rate of change in MAP. When MAP changed rapidly (0.35 mmHg/s), the CBF autoregulatory mechanism failed to operate and CBF followed the alteration of MAP. In view of this context, we designed our experiments so that the CoBF was measured continuously by the laser-Doppler flowmetry and MAP was slowly altered in a rampwise manner (0.25 mmHg/s) by

Fig. 7. The effect of intravenous injection of centrally active dose (2.5 nmol/kg) of naloxone, an opioid receptor antagonist, on CoBF autoregulation ($n=6$): Δ MAP shows changes in MAP. Δ BF shows % changes in blood flow. Open circles and triangles indicate responses of CoBF and MBF before the injection of naloxone, respectively. Closed circles and triangles indicate responses of CoBF and MBF after the injection of naloxone, respectively. Values are mean $\pm$ SEM.

inflation of the aortic occluder or the inferior vena caval occluder. Furthermore, the baroreceptor reflex may exert a significant influence on MAP-CBF relationship [19,20]. In the present study, therefore, to exclude the effect of the baroreceptor reflex on the autoregulatory maintenance of CoBF, all experiments were conducted on dogs with bilateral carotid sinus denervation plus vagotomy.

We found that the MBF was decreased with decreasing MAP and increased with increasing MAP. On the contrary, CoBF was well maintained at its normal level during changes in MAP (Fig. 4). Thus, the CoBF was subjected to strong autoregulation. Also in our experimental setting, responses of MBF to the changes in MAP were not altered by the intravenous injection of naloxone. On the contrary, the treatment impaired the CoBF autoregulatory mechanism (Fig. 4). This tendency was clearly observed during the decrease in MAP. These results indicate that endogenous opioids may participate in the CoBF autoregulatory mechanism. However, it is uncertain as to whether naloxone binds to peripheral or central opioid receptors because naloxone can pass through the blood-brain barrier due to its high lipid solubility [16, 17]. To examine this, the same dose of methyl

naloxone ($2.5 \mu\text{mol/kg}$), which does not cross the blood-brain barrier due to its greater polarity and reduced lipid solubility [16, 17] but has the same peripheral effects as naloxone [21], was intravenously injected instead of naloxone. We found that methyl naloxone did not alter the MAP-CoBF relationship (Fig. 5). Furthermore, the smaller dose of naloxone (2.5 nmol/kg) was injected into the cerebral ventricle. The response of CoBF to changes in MAP were enhanced by the intracerebroventricular injection of naloxone. Especially, this tendency was clearly observed during the decrease in MAP (Fig. 6). The effect of the intracerebroventricular injection of naloxone on the CoBF autoregulatory mechanism was similar to that induced by intravenous injection of naloxone. Furthermore, to exclude the possibility of leakage of naloxone to the systemic circulation, centrally active dose of naloxone (2.5 nmol/kg) was injected intravenously. The intravenous injection of smaller dose of naloxone did not affect the CoBF autoregulatory mechanism (Fig. 7). Thus, the intracerebroventricularly injected naloxone may exclusively bind to the central opioid receptors.

Overall, we conclude that endogenous opioids and central opioid receptors may be partly involved in the CoBF autoregulatory mechanism although we did not investigate the origin of endogenous opioids. Especially, the effects of naloxone were clearly shown during decreases in MAP. Several studies have demonstrated that the pituitary and adrenal glands release opioids into circulating blood in response to hemorrhagic hypotension [22–25]. Furthermore, Armstead *et al.* [22] showed that methionine-enkephalin, leucine-enkephalin, and dynorphin elicit pial arteriolar dilation. All of those lines of evidence in the literature suggests that secretion of opioids may be increased during decreases in MAP and that the opioids modulate cerebral vasodilation.

Naloxone acts as an antagonist of μ , κ , and δ opioid receptors. β -Endorphine, dynorphin, and enkephalins (methionine-enkephalin and leucine-enkephalin) have a strong affinity with μ , κ , and δ receptor, respectively. In the present study, we did not investigate which endogenous opioids and their corresponding receptors participate in the autoregulatory mechanism of CoBF.

CoBF autoregulation may be affected by endogenous opioids in the following two ways. The first possible mechanism is that endogenous opioids directly bind to opioid receptors located at the cerebral vascular smooth muscle cells, thereby modulating cerebral vascular resistance and CBF. Indeed, it was demonstrated that opioid receptors were located at the cerebral vascular smooth muscle cells or cerebral vessels in dogs [12], cats [26–28], and rats [29]. Furthermore, these studies also demonstrated that opioid compounds affected cerebral vascular resistance and CBF. The second possible mechanism is that activation of central opioid receptors may modulate cerebral vascular resistance by acting on the sympathetic [30] or on the parasympathetic [31] centers, whose neuron chain terminates in the cerebral vessels [7, 32, 33]. A previous study from our laboratory demonstrated that endogenous opioids dramatically decreased sympathetic nerve activity during hypotensive hemorrhage [34]. Furthermore, Nishimura *et al.* reported that a

medial septal microinjection of naloxone caused a significant increase in rat hippocampal blood flow [35]. It has also been suggested that endogenous opioids affect the local blood flow in the hippocampus, probably mediated by the cholinergic neurons projecting to the hippocampus. However, the vasodilator action of endogenous opioids are still controversial, since it is still unclear as to whether opioid compounds induce vasodilation [12, 26–28] or vasoconstriction [29, 35]. Indeed, in contrast with our findings, Armstead *et al.* [36] have shown that naloxone has no effect on regional CBF in normotensive and hypotensive piglets. These discrepancies may be due to species differences, regional differences, and/or differences of opioid receptor subtypes [37].

In conclusion, to examine the role of endogenous opioids in maintenance of CoBF, CoBF was measured continuously by employing the laser-Doppler flowmetry during changes in MAP. We found that CoBF was well maintained during changes in MAP, i.e., the CoBF was subjected to a strong autoregulation. Endogenous opioids and central opioid receptors may be partly involved in the CoBF autoregulatory mechanism. Especially, endogenous opioids may strongly modulate the autoregulatory cerebral vasodilation during the decrease in MAP.

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