Increases in Cerebral Blood Flow in Rat Hippocampus After Medial Septal Injection of Naloxone

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Background and Purpose: In a previous study, we occasionally found that the rat given naloxone in the preoptic region develops behavioral seizures. In view of knowledge that the forebrain including the medial septal nucleus provides cholinergic projections to the hippocampal formation, the present study examined the effects of naloxone injected into the medial septal nucleus on the local blood flow in the hippocampus.

Methods: A polyurethane-coated platinum electrode with a 1-mm bare tip for measurement of blood flow and a guide cannula made of stainless steel tube for naloxone injection were implanted chronically into the brain. The cerebral blood flow was measured by the hydrogen clearance method in freely moving rats.

Results: The injection of 50 μg naloxone caused a significant increase in hippocampal blood flow, with its peak at 20 minutes. Twenty micrograms naloxone caused a similar increase, but 10 μg caused only a slight increase that peaked at 30 minutes, suggesting a dose–response of naloxone effect. Hippocampal blood flow was not changed after the injection of saline into the medial septal nucleus and after the injection of naloxone into the caudate nucleus.

Conclusions: Taken together with previous findings, the results suggest that endogenous opioids exert a decreasing effect on the local blood flow in the hippocampus, probably mediated by the magnocellular cholinergic neurons projecting to the hippocampus. (Stroke 1992;23:1325–1330)

KEY WORDS • cerebral blood flow • hippocampus • rats • naloxone

In the course of a previous study in which we assessed the effects of naloxone injection in the preoptic region on the secretion of gonadotropin from the pituitary, it was occasionally found that rats given a naloxone injection exhibited behavioral seizures, which began approximately 15–20 minutes later and recurred intermittently at 15–20-minute intervals for about 24 hours.1 It was also found that such behavioral seizures accompanied epileptic spikings in the cortical electroencephalogram.

In the rat, the medial septal nucleus and the vertical limb of the diagonal band of Broca provide the major cholinergic projection to the hippocampal formation.2,3 It is known that stimulation of the medial septal nucleus enhances the release of acetylcholine from the hippocampus,4–7 and hippocampal neurons are excited by acetylcholine.8–12 The medial septal stimulation excites the hippocampal neuronal activity13 and induces after-discharges followed by kindled seizures.14 It appears possible that there is a mechanism in which the medial septal stimulation induces behavioral seizures by enhancing acetylcholine release and influencing the neuronal activity of the hippocampus. In support of this, the injection of carbachol into the hippocampus was able to induce kindled seizures.15 In view of this knowledge, it is suggested that, in our previous study, naloxone injection into the diagonal band of Broca somehow stimulated the release of acetylcholine in the hippocampus, enhanced the neuronal activity, and then produced electroencephalographic as well as behavioral seizures.

See Editorial Comment, p 1330

Recently, measurements of local cerebral blood flow (CBF) in localized areas have provided convincing evidence that changes in the neuronal function in one part of the brain are accompanied by appropriate changes in the local blood flow.16 We have shown, using the hydrogen clearance method devised for measuring local CBF in the freely moving rat,17 that the CBF in the hippocampus during the dark cycle is significantly greater than during the light cycle.18 It is known that the hippocampal electrical activity manifests excitation during the dark cycle.19–22 In addition, electrical stimulation of the medial septal nucleus has been shown to increase the CBF in the hippocampus in anesthetized rats.23 Therefore, we decided to investigate whether local CBF in the hippocampus would increase after the injection of naloxone into the medial septal nucleus or the diagonal band of Broca in freely moving rats.

Materials and Methods

Adult male Wistar rats weighing 250–350 g were maintained under controlled lighting (lights on 5 AM–7 PM) and temperature (24°C) and allowed free access to food and water. A 200-μm diameter polyurethane-coated platinum electrode (Unique Medical Co., Ltd., Tokyo, Japan) with a 1-mm bare tip was introduced
sterotaxically into the dorsal hippocampus in the right hemisphere under anesthesia with pentobarbital sodium at a dose of 31.5 mg/kg body wt, according to the coordinates of Albe-Fessard et al. 24 Electrodes were also implanted into the neocortex (the parietal cortex). A stainless steel screw was used as the reference electrode. A guide cannula made of stainless steel tube with a 0.65-mm outer diameter was also implanted stereotaxically for the microinjection of naloxone into the medial septal nucleus or diagonal band of Broca and the caudate nucleus. The inner cannula with a 0.30-mm outer diameter was arranged so as to protrude approximately 0.50 mm beyond the tip of the guide cannula. The guide cannula was plugged with a dummy inner cannula until the day of the experiment. The electrode and guide cannula were fixed to the skull with dental cement and three screws in the skull to serve as anchor posts for the dental cement.

The CBF was measured as described previously. 18 Briefly, a 15% hydrogen-85% air mixture was metered at a volume of 0.6 l/min for 3 minutes into the chamber in which the unanesthetized rat with the measuring electrode implanted was placed. Hydrogen washout curves were recorded on an X-Y recorder, and the CBF calculation was performed for the washout curve during the 2-minute period following the first 30 seconds with a small computer (Unique) based on the blood–tissue exchange theory of Kety and Schmidt.25

The measuring experiment was performed at least a week after the surgery between 9 AM and 6 PM, when the CBF in the hippocampus is considerably low and stable. 18 Each rat received injections of 2 μl saline at the first experiment and 50 μg naloxone (Sigma Chemical Co., St. Louis, Mo.) in 2 μl saline at the second experiment performed after 1 week. However, in the study to examine the dose response, animals received 0.5 μl saline at the first experiment and then 10, 20, and 50 μg naloxone in 0.5 μl saline in order of dose, at 1-week intervals.

On the day of experiments, the rats were placed in the chamber for at least 2 hours to adapt to the environment before the start of the experiment, with the leads connected to the reference electrode and the measuring electrode. Control CBF values were obtained by measuring the CBF three times, at 15–30 minute intervals, before the saline or naloxone injection. Saline or naloxone was injected for 2 minutes into the brain site of the unanesthetized rat. The CBF was measured at 10, 20, 30, 45, 60, 90, and 120 minutes after the injection. The measurement was stopped if behavioral seizures occurred, because hydrogen washout curves obtained during the seizures were not suitable for the calculation.

At the end of the experiment, the rats were anesthetized with sodium pentobarbital and perfused with 10% formalin. The brains were removed for histological identification of the sites of the electrode and cannula.

The CBF changes were expressed as percentages of the preinjection level obtained 10 minutes before the saline or naloxone injection. Means (±SEM) calculated for each measuring time were compared with the preinjection level by paired t test.

**Results**

Figure 1 shows the effects of the injection of naloxone in the medial septal nucleus or diagonal band of Broca on cerebral blood flow in hippocampus and neocortex, expressed as percentages of preinjection level obtained 10 minutes before injection. Number in parentheses is number of rats. Arrow indicates time of injection. Values are mean±SEM. *p<0.05, **p<0.01 compared with preinjection level (paired t test).

Figure 2 shows the effects of the injection of naloxone into the caudate nucleus on the CBF in the hippocampus. The locations of the 1-mm tip of the electrode in the hippocampus and neocortex are shown in Figure 3. The entire 1-mm-long electrode was located inside the hippocampus, i.e., the hippocampus proper and the dentate gyrus. The locations of the tip of the cannula in the medial septal nucleus or diagonal band of Broca and caudate nucleus are shown in Figure 4.

The injection of saline into the medial septal nucleus or diagonal band of Broca and the caudate nucleus did not produce significant changes in the CBF in either area during the 2-hour period of observation (Figures 1 and 2). The injection of naloxone resulted in an increase in the CBF in the hippocampus with its peak level at 20 minutes (85% increase, p<0.01 compared with the preinjection level), which was followed by a gradual decline, reaching the preinjection level 120 minutes after the injection (Figure 1). The CBF in the neocortex started to increase significantly at approximately 20 minutes and attained its peak (83% increase, p<0.05 compared with the preinjection level) at 45 minutes (Figure 1). The level remained relatively high thereafter during the period of observation. Further, there was no significant change in the CBF in the hippocampus after the injection of naloxone in the caudate nucleus over the 2-hour period of observation (Figure 2).
The effects of injection of various doses of naloxone into the medial septal nucleus or diagonal band of Broca on the CBF in the hippocampus are shown in Figure 5. The injection of 50 μg naloxone in 0.5 μl saline caused the CBF increase (50% increase, p<0.01 compared with the preinjection level), which peaked at 10 minutes, followed by significantly high levels until 90 minutes after the injection. The 20-μg dose of naloxone produced almost the same effect as that produced by 50 μg. The 10-μg dose produced a slight but significant increase (approximately 20% increase, p<0.05 compared with the preinjection level) only at 10 minutes. Therefore, 20 μg seemed the threshold dose for producing a pronounced CBF increase in the hippocampus by naloxone injection into the medial septal nucleus or diagonal band of Broca.

Discussion

The present study demonstrates that the local CBF in the hippocampus increases significantly after naloxone is injected into the medial septal nucleus or diagonal band of Broca. This CBF-increasing action of naloxone seems to have specificity for the injection site, since no increase in the CBF in the hippocampus was seen when injected into the caudate nucleus. Although the mechanism of action of this naloxone effect on the CBF in the hippocampus was not determined in the present study, the most reasonable explanation is that naloxone injected into the medial septal nucleus or diagonal band of Broca somehow activates the cell bodies of the cholinergic neurons located in these areas, resulting in the increased release of acetylcholine in the hippocampus, and then stimulates the chain of events leading to the rise of local blood flow in the hippocampus.16 As one strong piece of evidence, we have recently found that naloxone injected in the medial septal nucleus markedly enhances the release of acetylcholine in the hippocampus, as measured by the microdialysis method (T. Mizuno and F. Kimura, unpublished observation). Therefore, it is assumed that, in the medial septal nucleus, naloxone produces effects similar to those produced by electrical stimulation. Further support for this view comes from the findings that electrical stimulation of the medial septal nucleus enhances the release of acetylcholine in the hippocampus,4-7 stimulates the neuronal activity of the hippocampus,8-12 and causes an increase in the CBF in the hippocampus.23

However, an alternative explanation is that cholinergic neurons originating in the medial septal nucleus or the diagonal band of Broca are responsible for the vasodilation of vessels in the hippocampus. The ability of cerebral vessels to dilate in response to acetylcholine, either exogenously administered or endogenously released, has been extensively documented.26-28 Further, there is evidence that cerebral vessels are innervated by cholinergic nerves.29-31 Therefore, it is probable that an increase in the CBF in the hippocampus depends on this potent vasodilator substance, acetylcholine, but does not depend on an increase in metabolic activation...
caused by the neuronal activity, as has been recently proposed.23-32

The present results suggest that naloxone injected into the medial septal nucleus or diagonal band of Broca is able to stimulate the release of acetylcholine in the hippocampus, causing activation of neurons or dilation of vessels there. The mechanism for this is unclear, but there are a few possibilities. First, it is probable that cholinergic neurons are released from the tonic inhibition mediated by the endogenous opioids in the medial septal nucleus or diagonal band of Broca. It has been documented that the medial septal nucleus and diagonal band of Broca possess considerable amounts of \( \mu \)- and \( \kappa \)-receptors.33 In addition, there have been reports that an injection of \( \beta \)-endorphin into the medial septal nucleus inhibits acetylcholine turnover in the hippocampus,34 and this effect of \( \beta \)-endor-

phrin is mediated via the septal \( \gamma \)-aminobutyric acid (GABA) neurons,35 indicating that, in those cases, the receptor sites are at the level of the cholinergic cell bodies or on afferent terminals or interneurons innervating these cells.36

The second possibility is that naloxone antagonizes the GABAergic influence on cholinergic neurons in the medial septal nucleus and diagonal band of Broca and thus causes a disinhibition of cholinergic neurons. It is agreed that naloxone, especially at high doses, exerts GABA antagonistic effects aside from the potent opiate receptor antagonistic activity,37 and such a mechanism specifically accounts for the seizurogenic action of naloxone.36-40 However, this second possibility is less likely, since the experiments supporting naloxone as a GABA antagonist involved injecting naloxone systemically or iontophoretically into the olfactory tubercle, but not into the medial septal nucleus and the diagonal band of Broca.

The increase in cortical CBF with a considerable time lag after the injection of naloxone into the medial septal nucleus was interesting but was quite strange, because direct projections to the medial cortex originating in the medial septal nucleus and diagonal band of Broca have been described,41-43 in addition to those mediated via the hippocampus.44 The precise mechanism for this delayed response is unclear at present, although it is likely that the cortical effect occurred secondarily, fol-
ollowing the hippocampal effect, based on fiber connections mediated via the hippocampus. However, this finding seems to indicate, in turn, that the immediate increase observed in the CBF in the hippocampus is specifically produced, based on close fiber connections between the medial septal nucleus and the hippocampus, again showing the brain-site specificity for the naloxone effect. Although naloxone administered intravenously has been shown to block cerebral arterial vasocostrictive effects of noradrenalin and in high concentrations to produce vasodilatation and an increase in the CBF, the view of the brain-site specificity for the effect of naloxone appears to deny the possibility that the increase in the hippocampal CBF is produced by these rather nonspecific and direct effects on the cerebral vessels.

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