Intravenously and Iontophoretically Administered Naloxone Reverses Ischemic Changes in Rat Hippocampus

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Forty rats under urethane anesthesia were subjected to cerebral ischemia by ligation of the right carotid, the right plus the left carotid, or the right carotid plus two vertebral arteries. Ischemia caused three types of changes in the field potential of the right hippocampal CA1 region evoked by fimbrial stimulation: 1) completely reversible deterioration (57% and 16% of the rats with unilateral and bilateral carotid artery ligation, respectively), 2) moderate deterioration (37% and 24% of the rats with unilateral and bilateral carotid artery ligation) and 3) irreversible loss of the evoked activity (6% and 60% of the rats with unilateral and bilateral carotid artery ligation and all the rats subjected to three-vessel occlusion). Naloxone improved the moderate deterioration in 10 of 11 rats (1-3 mg/kg i.v.) and in 15 of 16 (50-150 nA) iontophoretic applications, but naloxone did not restore the lost evoked activity. Intravenous morphine (10 mg/kg) aggravated the ischemic changes, and this effect was reversed by naloxone, while iontophoretic administration of morphine caused only excitation. These findings suggest that naloxone has a favorable effect on cerebral ischemia not severe enough to cause transmission failure. The reversal of ischemic changes by iontophoretic naloxone indicates that its site of action is at the neuronal or microcirculatory level. (Stroke 1989;20:1059-1064)

Since the original report by Baskin and Hosobuchi that naloxone might reverse the effects of cerebral ischemia, the controversy about the role of naloxone and the opiates in cerebral ischemia has not been resolved despite numerous clinical1-11 and experimental12-26 studies. However, many anecdotal reports1-3,6-7,9-11 indicate that naloxone can cause a dramatic but usually temporary recovery of neurologic function. This effect, which can be reproduced by repeated injections, is not compatible with any known natural course of cerebral ischemic events and thus strongly suggests that naloxone may exert a favorable action on cerebral ischemia. This unique action of naloxone therefore deserves further research to evaluate its potential therapeutic role and to understand better the pathophysiology of cerebral ischemia.

Materials and Methods

We subjected 40 male Swiss albino rats weighing 200–300 g to anesthesia obtained by 1.2–1.5 g/kg i.p. urethane. Both carotid arteries were exposed following a ventral median surgical incision in the neck. The carotid arteries were separated from accompanying vagal and sympathetic nerves and suspended with 0 surgical silk sutures. To obtain arterial occlusion, using these sutures we pulled the carotid arteries into a glass tube and fixed the sutures by a clamp. The clamp was released and the glass tube was pulled back to restore blood flow. The vertebral arteries were cauterized using the method described by Pulsinelli and Brierley.27

After completion of surgery on the neck, we placed the rats in a stereotactic apparatus. The rectal temperature was monitored and maintained at 37–38°C with a radiant lamp. The heart rate was monitored by an electrocardiographic amplifier and a ratemeter. Stimulating and recording electrodes were lowered through burr holes in the skull to the appropriate sites, and the exposed surfaces of the cortex were subsequently covered with agar (3% wt:vol in physiological saline). Two parallel tungsten electrodes (tip separation 1.0 mm) were used for stimulation of the fimbria/commissure. The tips of the electrodes were lowered to a region with the following coordinates: anterior (A) 6.5, lateral (L) 0.5, height (H) 7.0 (according to the atlas of Albe-Fessard et al).28 Single-barrelled or multibarrelled
glass microelectrodes were used for recording the extracellular field potential and for microiontophoretic administration of drugs. The recording barrel was routinely filled with 3 M NaCl; when multibarrelled electrodes were used for iontophoretic applications, the other barrels were filled with naloxone HCl (Sigma Chemical Co., St. Louis, Missouri; 40 mM in 0.15 M saline at pH 6.0), morphine HCl (Verenidge, 40 mM in 0.15 M saline at pH 6.0), and saline (0.15 M at pH 6.0). The current applied for iontophoresis of drugs was automatically balanced with a current having the same magnitude but opposite polarity and was passed through the saline-containing barrel.

Positioning of the recording electrode in the CA1 region of the right hippocampus was guided by stereotactic coordinates (A 4.0, L 2.0–2.5, H ~2) and by observation of the characteristic field response evoked by fimbrial/commissural stimulation. Upon completion of the experiment, we perfused the rats with 10% formalin through a cannula in the left ventricle and removed their brains. The position of the stimulating electrodes was confirmed by minimal bleeding in the track and that of the recording electrode by the spot stained with pontamine blue (which had been iontophoresed through the recording channel after termination of recording in the CA1).

We administered the drugs systemically via a polyethylene catheter inserted into the jugular vein. Naloxone and morphine were prepared in physiological saline solutions at a 0.1 ml volume, which provides a dose of 1 mg/kg for a rat weighing 250 g.

Results

Stimulation of fimbrial/commissural fibers evokes a characteristic field potential in the CA1 region of the hippocampus; this potential consists of a posi-
Field potential recorded from CA1 region of right hippocampus of rat rapidly deteriorated and stabilized 3 minutes after ligation of ipsilateral carotid artery (RCA). Record B: Field potential recorded during 10th minute of ischemia, just before naloxone (NLX) injection. NLX restored field potential to great extent (C and D).

Naloxone in Cerebral Ischemia

The severity of the deterioration in electrical activity increased with the number of arteries ligated and was quite variable among rats. We obtained the most consistent findings in five rats, the vertebral arteries of which had been cauterized. Although normal field potentials could be recorded from these rats after cauterization, field responses rapidly and irreversibly disappeared following ligation of the ipsilateral carotid artery (Figure 1, A–D). The irreversible loss of the field potential was also observed in two of 35 rats after ligation of the ipsilateral carotid artery (the vertebral arteries intact) and in 15 of 25 rats in which both carotid arteries were ligated (Figure 1, E–H). Restitution of blood flow after 10 minutes did not restore the evoked activity in these rats (Figure 1H). We designated this kind of reaction to ischemia as a profound ischemic response. In another group of rats, deterioration of the field response was less pronounced and was completely reversible within 10 minutes despite the continued interruption of carotid blood flow (Figure 2, A–D). We observed this second type of reaction (a reversible ischemic response) in 20 of 35 rats in which the ipsilateral carotid arteries were ligated and in four of 25 rats in which both carotid arteries were clamped. In the rest of the rats subjected to ischemia, deterioration of the field response reached a maximum at around 1 minute and then partially recovered and remained stable after the third minute of ischemia (Figure 2, E–H, Figure 3B, Figure 4, B and C). Thirteen of 35 rats with ipsilateral carotid
was adequate, and no spontaneous recovery was observed. Naloxone (1 mg/kg) reversed the ischemic changes in seven of the 11 rats (Figure 3), an effect that was evident within the first minute of injection. Recovery of the field response was almost complete, with the reappearance of the population spike, but the spike had a lower amplitude (20-90% of control) and was slightly delayed (0.4-0.9 msec). The effect of naloxone lasted during the 30-60 minute follow-up. Escalating the dose of naloxone to 2 mg/kg in the rats in which the first injection was ineffective increased the number of responders to nine, and an additional injection of 1 mg/kg naloxone increased the number of responders to 10 (91%). Naloxone (1-3 mg/kg) did not appreciably modify the field response in four control rats who were not subjected to vessel occlusion or in the group exhibiting the reversible ischemic response.

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deterioration of the field potential, but these rats exhibited a powerful excitatory action of morphine.

Discussion

The failure of naloxone to reverse ischemia in some experimental and clinical studies is not surprising if one considers the heterogeneity of ischemic neuronal damage. It is likely that to demonstrate the beneficial effect of naloxone, neuronal damage must not have progressed to an irreversible stage and the functional reserve of at least some neurons must be close to the threshold for regaining electrical activity. Indeed, our finding that naloxone reverses the moderate ischemic response but not profound ischemic changes is compatible with the above view and suggests that naloxone could improve ischemic changes at a level before the failure of synaptic transmission.\(^{30-32}\)

Development of ischemia in the present model is an unexpected finding if one considers the well-known resistance to ischemia of rats subjected to ligation of only the carotid arteries.\(^{33-35}\) In fact, our initial intention was to use the four-vessel occlusion model.\(^{27}\) However, interruption of carotid artery blood flow following cauterization of vertebral arteries consistently led to irreversible loss of evoked activity. This sensitivity may have arisen from aggravation of ischemia by reduced systemic blood pressure due to urethane anesthesia\(^{26}\) and by the sensitivity of the electrophysiological method used as well as the vulnerability of the hippocampus to ischemia.\(^{37,38}\) Location of the hippocampal area in the watershed between the carotid and vertebrobasilar territories\(^{39}\) may have been a significant factor in making the CA1 region susceptible to the hemodynamic changes created in the present model.

Several previous articles reporting a beneficial effect of naloxone on cerebral ischemia studied the concomitant changes in cerebral blood flow,\(^ {20}\) blood pressure, and cardiac output.\(^ {6,9,11,14,20,23}\) and found no significant change in these parameters. Accordingly, naloxone was thought to act at the neuronal or microcirculatory level. Our data showing that iontophoretically applied naloxone can reverse ischemia-induced electrophysiological changes as well as does systemically administered naloxone agree with these reports and clearly demonstrate that naloxone can exert its anti-ischemic action without any contribution of cardiovascular factors or of the other regions of the brain. However, the data from iontophoretic experiments do not differentiate whether naloxone acts at the neuronal level or on the microenvironment of the neuron including capillaries and glial cells.

Is the anti-ischemic action of naloxone opiate-specific?\(^ {40-42}\) It has been reported that morphine exacerbated neurologic dysfunction in two patients who showed a favorable response to naloxone.\(^ {1}\) This observation has been confirmed in several experimental studies, and the action of morphine has been reported to be opiate-specific and independent of its effects on the cardiovascular and respiratory systems.\(^ {14,20,26}\) We also reproduced the unfavorable action of morphine on ischemia and showed this effect to be reversible by both intravenously and iontophoretically applied naloxone. The involvement of opiates at the cellular level could have been demonstrated convincingly by iontophoretic administration. Unfortunately, the well-known powerful excitatory action of iontophoretically administered morphine in the hippocampus\(^ {41,42}\) complicated the interpretation of changes in field potential. Yet it was clear that iontophoresis of large amounts of morphine (up to 200 nA) did not lead to a significant deterioration of the field potential. For example, a moderate ischemic response never progressed to an electrical silence after morphine iontophoresis.

In conclusion, our findings show that naloxone can reverse electrophysiologic effects of acute ischemia not severe enough to lead to total failure of evoked activity. The site of the anti-ischemic action of naloxone is probably at the neuronal level because local application of naloxone onto ischemic neurons was found to be as effective as its systemic administration. Although we found that intravenously applied morphine had an unfavorable effect on ischemia that was reversible by both systemically and iontophoretically applied naloxone, the opiate-specificity of naloxone’s action could not be demonstrated unequivocally at the neuronal level, possibly due to the powerful excitatory action of iontophoretically applied morphine in the hippocampus.

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