Failure of naloxone to affect focal incomplete cerebral ischemia and collateral blood flow in cats

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Focal incomplete cerebral ischemia was created in 20 adult cats by retro-orbital middle cerebral artery (MCA) occlusion under halothane anesthesia. Arterial blood gas, cerebral blood flow (CBF), bilateral electroencephalographic (EEG) recordings, and systemic arterial blood pressure (SABP) were monitored for the 1st hour of occlusion. Ten animals were treated with 10 mg/kg of naloxone within 10 minutes of MCA clipping, followed by a continuous infusion of naloxone at 2 mg/kg/hr for the duration of the occlusion (8 hours). Ten animals were treated in a similar fashion with physiological saline (control). Blood flow was restored after 8 hours. The brains were examined at the time of death or 7 days after the occlusion period. There was no difference between the two groups regarding cerebral infarction size or distribution, neurological outcome, SABP, PaCO₂, or CBF. Minor changes in EEG amplitude observed in the naloxone-treated group appear to represent interaction of the drug with halothane after prolonged administration. The authors conclude that naloxone did not modify the outcome of focal cerebral ischemia in the cat.

Key Words • naloxone • cerebral ischemia • cerebral blood flow • arterial occlusion • cerebral infarction

NALOXONE, a competitive opioid receptor antagonist, has been reported to improve functional recovery after experimental spinal cord trauma in cats, possibly by directly increasing cord blood flow. It has also been reported to temporarily reverse ischemic deficits in gerbils and in man, although the mechanism of action was not elucidated. It is tempting to postulate that this is due to a drug-induced improvement in cerebral blood flow (CBF) in the “ischemic penumbra,” allowing an electrically silent but viable area of cerebrum to become functional. However, no one has demonstrated histological evidence of reduction in infarction size, CBF, or electroencephalographic (EEG) responses to naloxone in models of cerebral ischemia.

If naloxone is beneficial, it may exert its effects by direct neuronal action (membrane stabilization by opioid receptor or nonopioid receptor antagonism), or indirectly by increasing CBF above the critical ischemic threshold. We have designed an experiment to determine if naloxone reduces infarction size following temporary unilateral middle cerebral artery (MCA) occlusion, and to determine how its effect is mediated. The cat is a well established model for focal cerebral ischemia, and ideally suited to this study because of its potential collateral circulation.

Materials and Methods

Experimental Design

Twenty-eight unselected adult cats of both sexes, each weighing 2.5 to 4.0 kg, were used in the experiment. Halothane was the sole anesthetic agent used in order to minimize potential drug interactions. The right MCA was atraumatically occluded for 8 hours. During at least 6 of the 8 hours, the animal was awake and breathing spontaneously. Each cat was prepared in an identical fashion as detailed below, except for randomization to treatment with naloxone or physiological saline after MCA occlusion. Criteria for inclusion in the two treatment groups were: 1) a technically adequate EEG; 2) visually observed occlusion of the MCA by a vascular clip; and 3) EEG evidence of focal ischemia as demonstrated by a significant amplitude depression in the right frontotemporal channel. The latter was at least a 20% reduction in amplitude as compared with the preocclusion (baseline) value in each animal.

Experimental Technique

Each animal was anesthetized with 4% halothane, orally intubated, and subjected to artificial ventilation. The maintenance inspired halothane concentration was
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FIG. 1. Schematic representation of a coronal section anterior to the optic chiasm of a cat. The gamma detector is shown positioned over the zygoma, and the composite areas of infarction are identified by stippling. The superior and inferior orbital rims, mandibular process, and overlying temporalis muscle are shown on the right.

1.28% to 1.37%, confirmed by a halothane analyzer. This provided an adequate level of anesthesia for surgery and control of the respiratory rate. No muscle relaxants or other agents were used except heparin flushes.

Right femoral artery, femoral vein, and lingual artery catheters were placed and the cat secured in an atraumatic head holder. A cruciate scalp incision was made and the temporalis muscle reflected bilaterally. A right retro-orbital craniectomy was performed, and the right MCA freed from its arachnoid adhesions near its origin. Three gold-plated 1.2-mm EEG electrodes were implanted epidurally over each hemisphere in a uniform fashion, allowing continuous monitoring of the EEG (four channels: left and right frontotemporal, left and right frontoparietal). A partial craniectomy was made just above the junction of the right squamous and coronal sutures for positioning of a 1.5-mm solid-state gamma detector† (Fig. 1).

The right MCA was visually occluded close to its origin with a vascular occlusion clip‡. Ten minutes after occlusion, either naloxone (1 ml/kg of a 10-mg/ml solution) in saline (10 mg/kg), or physiological saline (control, 1 ml/kg), was given as a bolus through the femoral vein catheter. This was followed immediately by a continuous infusion of intravenous naloxone (2 mg/kg/hr) or an equal volume of saline (1 ml/kg/hr) by means of an infusion pump.

After 1 hour of occlusion, all monitoring lines were removed, except the femoral vein catheter for continued administration of treatment solutions. A suture attached to the free end of the occluding vascular clip was brought out through the scalp and the wounds closed. Flo-cillin (75,000 units) was administered intramuscularly, and the cat allowed to awaken from anesthesia in an atraumatic animal restraint. The total anesthesia time from the point of MCA occlusion averaged 1.89 hours.

At the end of 8 hours of occlusion, the vascular clip was gently withdrawn from the artery by traction on the protruding suture. The intravenous infusion of saline or naloxone was stopped, and the catheter removed. These procedures were facilitated by administering light halothane anesthesia by facemask. The animal was allowed to recover and was given free access to food and water. At the time of death or at 7 days postoperatively, the brain was removed and fixed in 10% formalin (Fig. 2).

Measurements

The systemic arterial blood pressure (SABP) and four-channel EEG were recorded continuously. Arterial blood gas (ABG) and CBF measurements were determined at standard intervals. The ventilator and inspired gases were adjusted to maintain a constant pCO2 between 35 and 42 torr. The CBF was calculated by the

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† Gamma detector manufactured by Radiation Monitoring Devices, Watertown, Massachusetts.
‡ Kees temporary clip, 5 mm length, 10 to 15 gm closing pressure, manufactured by Kees Surgical Specialty Co., Wil- der, Kentucky.
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FIG. 3. Posterior view of right middle cerebral artery infarction after standard sectioning of cat brain.

initial slope method of xenon washout\(^2\) after 450 \(\mu\text{Ci}\) of xenon-133 (\(^{133}\text{Xe}\), Xeneisol) was injected through the right lingual artery catheter. Measurements of CBF and ABG were made after exposure of the right MCA (baseline), 5 minutes after occlusion of the MCA (pretreatment), and 1 hour after occlusion (posttreatment). A neurological evaluation was performed after removal of the vascular clip at 8 hours, and daily for 7 days, based on the level of consciousness and the presence of weakness and forced motor activity.\(^{13,25}\) The formalin-fixed brains were transversely sectioned at the level of the obex, and the volume determined. Each brain was sectioned in a miter box and the volume of infarction calculated with a planimeter\(^4\) (Fig. 3). Histological sections were made in cats with small or no grossly apparent infarcts. The extent of infarction observed grossly correlated well with the microscopic findings.

The mean arterial blood pressure (MABP) was calculated from the continuous record. The average EEG amplitude and frequency were determined at standard intervals in the right and left frontotemporal channels. The results were subjected to statistical analysis. Probability less than or equal to 0.05 (\(p \leq 0.05\)) was considered significant.

Results

Four cats had no significant change in the EEG despite adequate visual occlusion. Two animals died after anesthetic and less than 6 hours of occlusion, one while receiving naloxone and the other saline. The naloxone-treated animal had a witnessed generalized seizure followed by a respiratory arrest, and the control animal had an unwitnessed respiratory arrest (?seizure). Neither animal had gross or histological evidence of hemorrhage, infarction, or edema, and they were omitted from the study group. One animal was omitted for a technically inadequate EEG, and one cat was found to have a chronic nonobstructive hydrocephalus and cystic cerebellum at autopsy. Hence, 20 animals were included in the study group, 10 receiving naloxone and 10 saline after MCA occlusion.

Infarct Size

Nine of 10 cats in each treatment group had an infarction in the right MCA distribution. The mean infarct size, expressed as percent of whole brain volume, was 3.14% \(\pm\) 0.96% in the naloxone group and 4.65% \(\pm\) 1.47% in the control group (Fig. 4). This did not reach significance (\(p = 0.25\)). The only evidence of hemorrhagic infarction occurred in the head of the right caudate nucleus in one naloxone-treated cat and three control animals. This generally occurred within a larger ischemic infarct. The distribution of the infarcts (which included the caudate nucleus, with possible additional involvement of the internal capsule and cortex) was very similar in both groups (Fig. 1).

Neurological Outcome

There was no difference between the treatment groups in mortality, focal weakness, circling to the right (forced motor activity), or level of consciousness. Two cats in the naloxone group and three in the control group died within 24 hours of MCA occlusion. All exhibited severe hemiparesis and were unable to stand. All had large (7.05% to 10.29%) infarcts, grossly extending in a wedge-shaped fashion from the caudate nucleus to the cortex. Only one of the five (a control animal) hemorrhaged into the caudate nucleus.

Upon recovery from anesthesia 1\(\frac{1}{2}\) hours after occlusion, decreased left upper-extremity withdrawal in re-
sponse to noxious stimuli was noted in most animals, but further testing was not possible due to the limitations of the animal restraint. A decline in the level of spontaneous activity was generally noted 3 to 4 hours after occlusion.

The neurological deficits were maximal in all animals within 24 hours of MCA clipping. Only one cat (a control animal) that survived more than 24 hours deteriorated in level of consciousness and died on postoperative Day 3. In this animal, herniation was not noted but hemorrhage into the caudate nucleus as well as caudate, capsular, and cortical ischemic infarction were present. The overall distribution of infarctions and its correlation with the animals' neurological deficits agrees with the report by Hayakawa and Waltz of MCA occlusion in awake cats.

One animal from each group had no microscopic evidence of infarction. Both cats were neurologically intact, and exhibited normal pacing activity in their cages.

Cerebral Blood Flow

The mean CBF values are shown in Fig. 5. While there tended to be a reduction in collateral flow following ipsilateral MCA occlusion (pretreatment), this was not significantly different from the baseline value. The posttreatment CBF was not different from baseline within each treatment group. The apparent difference in the posttreatment CBF between therapeutic groups is a manifestation of the variation in baseline CBF, and no significant difference was found (p = 0.25) after intensive statistical analysis.

There was no difference between treatment groups in MABP or partial pressure of arterial carbon dioxide (PaCO₂) at the three measurement points except for the baseline MABP (Fig. 5). There was no change in MABP with the naloxone bolus, in agreement with Young and colleagues but in disagreement with others. This discrepancy may be due to the interaction of naloxone with neuromuscular blocking agents used in the latter experiments.

Electroencephalographic Effects

By our criteria for inclusion in the study groups, the amplitude of the right frontotemporal channel was significantly decreased from baseline after right MCA occlusion (Fig. 6). This postocclusion (pretreatment) amplitude, expressed as percentage of baseline amplitude, was 57.7% ± 4.7% (p < 0.001) in the naloxone group and 56.5% ± 5.3% (p < 0.001) in the control group. At the posttreatment point, the amplitude in the control group remained depressed at 47.2% ± 4.8%, not significantly changed from pretreatment levels. While the naloxone-treated group remained depressed at 70.2% ± 5.4% of the baseline amplitude, the 13.5% improvement from the pretreatment amplitude was significant (p = 0.035).

The baseline EEG frequency was 6 to 8 Hz, slowing to 2 to 3 Hz in the right frontotemporal lead in both groups after MCA occlusion. The frequency remained slow despite either treatment.

The left frontotemporal channel was examined for evidence of diaschisis. There was no significant change in amplitude or frequency following contralateral MCA occlusion in either group (pretreatment). An 8% to 12% decrease in amplitude was noted in both treatment groups by the end of the monitoring period, although frequency was unchanged.
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FIG. 6. Representative four-channel electroencephalogram demonstrating unilateral slowing and depression of amplitude following right middle cerebral artery occlusion. F = frontal; T = temporal; P = parietal; R = right; L = left.

Discussion

Reliability of Technique

The cat is a widely studied well established model for focal cerebral ischemia. Cerebral infarction in the distribution of the MCA is reliably produced with permanent occlusion of that vessel. The infarctions in cats result in a low postoperative mortality compared with similar occlusions in primates and with the Mongolian gerbil model, allowing chronic studies to be performed. Two approaches to the proximal MCA are favored, the retro-orbital and the trans-orbital, both allowing good exposure with minimal brain-tissue manipulation.

In preliminary trials, we determined that a temporary vascular clip with a low closing pressure could be withdrawn from a partially dissected MCA by the technique described without causing avulsion of perforators or subarachnoid hemorrhage. In sham-operated animals, no focal EEG change occurred from operative manipulation alone.

One major objection to the cat model for studying stroke is the variation in size of the infarction resulting from proximal cerebral artery occlusion. This is thought to be due to the ability of leptomeningeal anastomoses between major arteries to compensate for acute occlusion. We have demonstrated this, for despite marked impairment of electrical activity after MCA occlusion, recovery without infarction was possible in either treatment group. In cats, a threshold CBF for electrical (synaptic transmission) failure and a lower critical threshold for failure of ion homeostasis similar to that in baboons has been demonstrated. Expanding the concept of the ischemic penumbra, we postulate that after focal arterial occlusion, this ischemic zone of reversibility will be larger in the cat model than in the primate because of the better collateral circulation in the former (see below). A treatment that improves CBF should show the greatest reduction in infarct size in the model with the largest zone of still viable tissue at risk.

Ischemic Tolerance

The ischemic tolerance of neural tissue in focal incomplete cerebral ischemia varies with many factors. The control of systemic factors such as MABP, pO\textsubscript{2}, and pCO\textsubscript{2}, as well as blood volume and hemodilution, is critical in determining the effects of exogenous agents on stroke. It has been clearly demonstrated that autoregulation of CBF breaks down in the ischemic cerebrum and CBF becomes directly dependent on MABP. At low CBF, approaching the critical threshold of ischemia, maintenance of electrical cortical activity and ion homeostasis can be directly manipulated by adjustment of MABP. If CBF falls below the threshold for electrical activity in the motor cortex, but remains above the ion “pump” threshold, a neurological deficit will result but the cortex will remain viable. If blood flow is increased into the affected area by surgery, by other therapeutic measures, or by a gradual improvement in the collateral circulation, neurological recovery will occur. Frequently, in the cat model, rapid recovery will occur during a time when cerebral edema should be maximal, as witnessed in this study and by others. The choice of anesthetic agents can also affect the ischemic tolerance, independent of effects on general systemic factors. This complicates the evaluation of treatments instituted while the animal is anesthetized. In the awake cat, neurological deficits occur within several minutes of acute MCA occlusion.
known what the minimum occlusion time is for infarction to develop in the awake animal. In this study, it appeared that a minimum of 6 hours of occlusion, with approximately 5 of these hours in the unanesthetized state, is necessary for infarction to be produced in 90% of cats. In the barbiturate-anesthetized cat, MCA occlusion can be tolerated for at least 6 hours without infarction if the animals are treated with high-dose barbiturates shortly after occlusion. It has been suggested that barbiturates are "protective" by lowering cerebral metabolic demands by inhibition of synaptic transmission.

The true effects of naloxone or any other agent being tested might be obscured by a similar protective effect of barbiturate in both the treated and untreated groups.

The advantages of using halothane in this model are: 1) a constant level of anesthesia can be maintained throughout the monitoring period and applied uniformly to each test subject; 2) an anesthetic level similar to that used clinically in humans can be attained without resorting to paralyzing agents; 3) there does not appear to be any appreciable reversal of halothane anesthetic by naloxone, as determined by a change in cerebral metabolic rate; and 4) the anesthetic can be quickly reversed to allow for early neurological evaluation.

Cerebral Blood Flow Measurements

The true severity of focal incomplete cerebral ischemia in the cat is not reflected using $^{133}$Xe CBF determinations because of the "look through" phenomenon and the deep location of the area of maximal ischemia. The "look through" phenomenon results from a failure to deliver xenon into the area of ischemia, so the gamma detector records counts only from regions deep and adjacent to the area of ischemia, thus "looking through" the region in which flow has been reduced to such a critical level that a representative amount of $^{133}$Xe has not been delivered for a measurement. Better collimation of the sodium iodide crystal detectors helps to reduce this artifact but does not eliminate it, even in humans with a relatively small detector for the brain size. In fact, the only occasion in which true ischemia is reflected by $^{133}$Xe CBF measurements is the unique situation achieved during carotid endarterectomy in which the indicator is delivered to the area predestined for ischemia immediately prior to carotid artery occlusion. In this instance, the clearance of the indicator is a true reflection of collateral flow and the accuracy of the technique is well established. In the laboratory preparation, the constellation of a small brain, relatively large probe, deep infarction, and poor spatial resolution characteristics of xenon makes it extraordinarily difficult to measure accurately the true severity of focal ischemia.

The solid-state detector used in this study was placed over the skull at a point where collateral CBF was measured rather than CBF in the area of focal ischemia (Fig. 1). Waltz demonstrated a fall in CBF after MCA occlusion using krypton-85 in the halothane-anesthetized cat, so flow reduction in the overlying cortex does occur (krypton-85 is a beta-emitter and thus free of artifacts related to the "look through" phenomenon). However, we detected no significant change in mean collateral CBF with MCA occlusion despite a marked depression in the EEG (see Hossmann and Schuier). We attribute this discrepancy to the indicator employed. Nevertheless, sequential measurements within the same animal are accurate for comparative purposes and they demonstrate no apparent increase in collateral flow from naloxone.

Electroencephalographic Alterations

The EEG amplitude and frequency was abruptly depressed ipsilateral to the MCA occlusion in 24 of 28 cats tested, without evidence of diaschisis. These findings and the degree of amplitude reduction are in agreement with the observations of Hossmann and Schuier. Unlike Hossmann, we found concomitant slowing in frequency (Fig. 4), which was also documented by Waltz; this slowing was similar to that described in humans undergoing carotid endarterectomy whose flows fall below the electrical threshold. In our ischemic animals, we did not observe further EEG alterations after the bolus of naloxone such as those described by Artru and colleagues in normal dogs under halothane anesthesia. There was a 13% to 15% increase in the depressed amplitude (p = 0.035) and frequency (p = 0.18) at the posttreatment point in the naloxone group. On the other hand, the initially unaffected left frontotemporal channel showed a gradual decrease in amplitude by 8% to 12% in both groups at the posttreatment measurement, without alteration of frequency.

Whether this represented some recovery of neuronal activity on the ischemic side or an effect of the naloxone on the anesthetic agent could not be determined. We therefore tested a sham-operated animal, the right MCA exposed but not occluded, maintained at 1.3% halothane concentration. The baseline frequency was 8 to 12 Hz. The EEG amplitude decreased for 1½ minutes in all four channels a few seconds after a 10-mg/kg naloxone bolus, which agrees with the transient EEG "reversal" seen in the dog. The MABP was not affected. After 45 minutes of 2 mg/kg/hr naloxone infusion, intermittent bursts of high-amplitude activity at 6 to 8 Hz were noted, and by 60 minutes the dominant pattern was 4 to 6 Hz. Amplitude at 60 minutes was 110% of the pretreatment value in all channels. Thus, it appears that prolonged administration of naloxone results in an EEG pattern of deeper halothane anesthesia without blood pressure change. Why this effect is skewed in the cat with unilateral ischemia is not clear.

There were periodic spontaneous movements of the extremities of sham-operated cats after naloxone bolus and infusion was begun, but these did not correlate with a change in the EEG.
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Gerbil Model

Due to an incomplete circle of Willis in approximately 40% of Mongolian gerbils, unilateral occlusion of a common carotid artery in this group results in ischemic cerebral symptoms.15,22 The 40% of animals that develop neurological deficits is a heterogeneous group, with some animals showing symptoms within 30 minutes of occlusion and others displaying a progressive deficit only after hours of occlusion. Seizures were observed by Yanagihara28 in 75% of symptomatic gerbils within 3 to 12 hours of occlusion and were documented by EEG.

Gross evidence of cerebral infarction is evident in the more severely affected animals within 3 hours of occlusion, and the animals in this subgroup are dead or comatose by 24 hours.28 The variation in infarction size and neurological outcome is related to the degree of anastomoses present (and hence the degree of impairment of CBF) and is also related to whether seizures are present. Due to the small size of the animal, it is technically difficult to obtain MABP and pCO₂ measurements. Without this information, and without EEG monitoring or an assessment of CBF, the interpretation of the effects of naloxone, morphine sulfate, or other agents is difficult. Indeed, a recent paper14 casts doubt on the ability of naloxone to influence recovery after unilateral carotid ligation in gerbils, conflicting with the report by Hosobuchi, et al.15 on this model.

Pharmacology of Naloxone

Naloxone is a stereospecific competitive opioid receptor antagonist, nearly devoid of any agonist properties in normal man, even in massive doses.17 Opioid receptors are a heterogeneous population, with multiple subtypes for which naloxone has varying affinity.18 This variation in affinity has been invoked to explain the discrepancy between the lack of effect of naloxone at 1 mg/kg dosage and the apparent benefit at 10 mg/kg after spinal cord contusion in cats.1,12,19 Alternatively, naloxone may act through nonopioid receptor mechanisms such as membrane stabilization.11

Dosages of naloxone that have appeared beneficial in animal experiments would be the equivalent of 70 to 700 mg in the average man.10,11,13,29 Yet, Baskin and Hosobuchi29 have described the improvement in neurological deficits presumed secondary to cerebral ischemia in three human patients with 0.4-mg boluses. This is difficult to reconcile unless marked interspecies differences in opioid receptor affinity exist.

Peak levels of naloxone are attained in the serum and brain within 15 minutes of administration.19 The drug crosses readily into the central nervous system (CNS), with brain/plasma ratios of 2 to 4/1 and a CNS half-life of 40 to 60 minutes in rats.6,19 The duration of action in man is 1 to 4 hours, proportional to the dosage.17 The dosage and timing of administration of naloxone in this study should achieve a peak CNS level within 25 minutes of occlusion and it should be maintained for the duration of the ischemic period.

While moderate doses of naloxone have little effect on normal man, they may be effective in states with abnormal elevated endogenous opioid systems.5,7 The pathophysiology is not clear. It has been suggested that endogenous opioids may serve neurotransmitter and neurohormonal functions.18 Alternatively, naloxone acts by nonopioid receptor mechanisms to antagonize γ-aminobutyric acid (GABA)-evoked inhibition of neuronal firing in rat brains, provoking clonic seizures.9 It might, therefore, demonstrate arousal or reversal of neurological deficit by acting as a GABA antagonist. Again, this underscores the need for careful monitoring of physiological parameters when evaluating naloxone's multisystem effects.

Conclusions

Naloxone had no effect in modifying cerebral infarction size in cats following temporary unilateral MCA occlusion. The MABP and pCO₂ were not affected, and no significant change in collateral CBF around the area of ischemia could be detected. Prior to treatment, EEG monitoring revealed similar ipsilateral amplitude and frequency depression in both the control and naloxone groups, reflecting similar regional ischemia. A partial "recovery" of the EEG in the naloxone-treated group appears to indicate interaction of the drug with halothane, and the mechanism by which this occurs is unknown.

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