β-Endorphin in Experimental Canine Spinal Ischemia

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Plasma and cerebrospinal fluid β-endorphin concentrations were radioimmunologically assayed in dogs subjected to spinal cord ischemia induced by infrarenal aortic ligature and in control sham-operated dogs. Plasma β-endorphin levels rose significantly following surgery in control dogs but were unaffected by spinal ischemia. On the other hand, a significant increase in cerebrospinal fluid β-endorphin concentration occurred after spinal ischemia, while surgical stress had no significant effect. Thus, the origins of plasma and cerebrospinal fluid β-endorphin may be different, with the former secreted from the hypophysis and the latter from nervous tissue. Observed changes in cerebrospinal fluid β-endorphin concentration could be related to the ischemic lesion of nervous tissue while the changes in plasma levels may reflect general stressing factors such as the surgery in our experiments. (Stroke 1989;20:253-258)

It has been proposed that endogenous opioids, mainly β-endorphin, might play a pathophysiologic role in cerebral ischemia since the opiate antagonist naloxone improves neurologic function following carotid occlusion in gerbils. Moreover, naloxone in low dosages completely reversed the neurologic deficits in two patients with cerebral ischemia, whereas intravenous morphine sulfate profoundly exacerbated the hemiparesis in one of these patients. For these reasons and due to the fact that endorphins have a depressive effect on motor activity, it has been suggested that cerebral ischemia may cause an increase in the β-endorphin concentration that, in turn, aggravates the neurologic deficit through an unknown mechanism. In some studies of experimental cerebral ischemia researchers have failed to reproduce the beneficial effect of naloxone, and subsequent clinical studies were either unable to confirm it or confirmed it only partially. β-Endorphin concentration was found to be increased in the cerebrospinal fluid (CSF) of the two patients suffering from acute cerebral ischemia. However, these were postoperative patients who had had intracranial surgery and had exhibited diminished levels of consciousness. A significant increase of plasma β-endorphin concentration has also been detected in cats 30 minutes after acute experimental cervical trauma, but spinal shock was also present, which might better explain the increase in β-endorphin concentration. However, naloxone administration improved the cats' survival and spinal blood flow and minimized neurologic deficits.

Recent animal studies have shown that naloxone improves physiologic parameters in canine embolic stroke, and clinical and functional observations of patients with acute spinal cord injuries have shown similar improvement with naloxone, although this last study was not statistically verified. Nevertheless, direct evidence of endorphin involvement in ischemic lesions of nervous tissue is still lacking.

Our aim was to measure β-endorphin concentrations in both CSF and plasma before, immediately after, and for 48 hours during experimental spinal ischemia in dogs.

Materials and Methods

Fifteen adult mongrel dogs, fasted for 12 hours before surgery, were anesthetized with 30 mg/kg i.v. thiopental sodium and 4 mg/kg i.v. ketamine.
Catheters were inserted percutaneously into the cisterna magna and into the right carotid artery for sampling of CSF and blood. Arterial blood pressure was monitored for approximately 2 hours with a Statham transducer (Cleveland, Ohio) during the acute stage of the experiment. Ten dogs were subjected to spinal lumbar ischemia by tying the abdominal aorta, via a retroperitoneal approach, just below the origin of the renal arteries. In five control dogs the snare was inserted but not tightened. Surgery lasted approximately 30 minutes. Hind limb surgery lasted approximately 30 minutes. During the 6 hours following surgery, all dogs received normal saline with 5% glucose by drip; thereafter they were allowed to move, eat, and drink freely.

Complete recovery from anesthesia occurred approximately 2 hours after surgery. Hind limb neurologic impairment was graded twice daily for 48 hours by two investigators on a four-point scale (0: complete paralysis, muscle flaccidity, tendon reflexes absent, pain sensibility absent, incontinence for urine and feces; I: some functional movement retained, muscle hypertonicity, reflexes exaggerated, pain sensibility present, sphincter control variable; II: ability to stand and walk a few steps, muscle hypertonicity, reflexes exaggerated, pain sensibility present, sphincter control normal; and III: normal).

CSF (1 ml) and blood (5 ml) samples were drawn simultaneously when the appropriate level of surgical anesthesia was reached (time -0.5) and 0.5, 1, 2, 3, 6, 12, 24, and 48 hours after experimental or sham aortic ligature. Immediately after collection, CSF samples were mixed with acetic acid for 10 minutes at 100°C, lyophilized,14 and assayed by radioimmunoassay (RIA). Blood samples were drawn into borosilicate glass tubes containing 1 mg/ml EDTA and 5 × 10^7 IU/ml Trasylol (Bayer, Leverkusen, FRG); plasma was separated in a refrigerated centrifuge and stored at -20°C. Two and one half milliliters of a vigorously mixed suspension of anti-β-endorphin particles adsorbed onto Sepharose15 were put into columns packed with Sepharose, allowing the supernatant to run through using a rubber bulb; then the plasma samples were added. The columns were stoppered and then rotated for 4 hours at 4°C. Afterwards, the plasma was drained through the columns, which were washed three times with 1-ml aliquots of 0.85% saline. Finally, the β-endorphin was eluted twice with 250 μl of 0.025N HCl, and the combined eluate was immediately assayed. β-Endorphin in both the CSF and the plasma was measured after extraction by a double-antibody RIA method (Immunonuclear Co., Stillwater, Minnesota). Using this method, the antibodies to β-endorphin demonstrated 5% cross-reactivity with β-lipotropin and no cross-reactivity with other peptides or hormones.

Normal baseline concentrations had been established in healthy unanesthetized dogs that were fasted and allowed to rest for 12 hours before CSF and blood sampling (data not included). Mean±SD concentrations of β-endorphin in CSF and blood were 100±55.8 (range 48–178) and 45.6±31.4 (range 8–60) pg/ml, respectively. Statistical evaluation was performed by means of analysis of variance.

Four days after the end of the experiment, all 15 dogs were killed with a barbiturate overdose. The spinal cord was then removed and fixed in alcohol. To measure the cranio-caudal extent of the lesion, 10-μm cross-sections were taken at 2-mm intervals from the lower thoracic region to the conus medullaris. The slices were stained using Nissl’s method for the neurons and the Marsland-Glees Erikson method modified by Winckler for the axis cylinders. The tissue was examined by a histologist unaware of the clinical status of the dogs. Spinal cord lesions were graded on a four-point scale (0: liquefaction of gray matter, degeneration of fibers; I: anterior horn necrosis, demarcation; II: multifocal neuronal loss, gliosis; and III: normal).

### Results

Six ligated dogs had a neurologic impairment score of 0 and four had a score of 1; only the five control dogs had a score of III. The results are reported in Table 1.

<table>
<thead>
<tr>
<th>Neurologic impairment</th>
<th>Dogs</th>
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<tbody>
<tr>
<td>Score</td>
<td>Definition</td>
</tr>
<tr>
<td>0</td>
<td>Complete paralysis, muscle flaccidity, tendon reflexes absent, pain sensibility absent, incontinence for urine and feces.</td>
</tr>
<tr>
<td>I</td>
<td>Some functional movement retained, muscle hypertonicity, reflexes exaggerated, pain sensibility present, sphincter control variable.</td>
</tr>
<tr>
<td>II</td>
<td>Ability to stand and walk a few steps, muscle hypertonicity, reflexes exaggerated, pain sensibility present, sphincter control normal.</td>
</tr>
<tr>
<td>III</td>
<td>Normal</td>
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Microscopic evaluation of the spinal cord showed pathologic changes in all 10 ligated dogs, with a transition zone at the upper lumbar segment levels. Nissl’s stain for neurons demonstrated that the ischemic lesion involved the anterior horns and the
intermediate gray matter around the ependymal channel. Marsland-Glees Erikson stain for axis cylinders showed either minimal or no evidence of myelin breakdown in the long white matter tracts, whereas fibers emerging from the anterior horns were totally or partially destroyed. Spinal cord lesions graded according to the four-point scale are reported in Table 2.

The correlation between neurologic deficits and neuropathology was satisfactory. All dogs showing marked neurologic impairments (Table 1, score 0) had more extensive spinal cord lesions (Table 2, score I), whereas dogs with less neurologic impairment (Table 1, score II) showed minimal spinal cord lesions confined mainly to the lumbar segment (Table 2, score II). Control dogs showed no impairment and normal histopathology.

Arterial blood pressure did not change during the 2 hours after abdominal aorta ligation.

In the CSF of ligated dogs β-endorphin concentration remained within the normal range when the appropriate level of surgical anesthesia was reached. A rapid rise in opioid concentration occurred 0.5 hours after the spinal stroke, with a mean±SD peak of 315.4±88.3 pg/ml, significantly greater than the values measured during anesthesia alone (132±59.1 pg/ml). At 1 hour after the stroke, β-endorphin concentrations fell, but not to below the normal range. A further rise occurred by the second hour, and the concentration remained high until the 12th hour, returning to the anesthesia value by the 24th hour, and remaining stable thereafter (Figure 1, top). In the control dogs, the CSF β-endorphin concentration was also unmodified by anesthesia; the concentration rose slowly, with a peak at the third hour (154±58.7 pg/ml) followed by a gradual decrease with return to the anesthesia value from the 12th hour thereafter; these differences were not significant (Figure 1, top).

Among the ligated dogs, trends in β-endorphin concentration were similar in the dogs with severe (score 0) and those with slighter (score II) neurologic deficits. However, in the latter group, the increase in opioid concentration was smaller, though not significantly so (Figure 1, bottom).

In the plasma of ligated dogs, β-endorphin concentration increased when the appropriate level of surgical anesthesia was reached; however, this rise was not significant. Plasma opioid concentration showed a further increase at 0.5 hour after the spinal stroke, with a mean±SD peak of 114.6±45.4 pg/ml, significantly greater than both baseline and values during anesthesia. β-Endorphin concentration then fell to 56.8±29.6 pg/ml at the third hour, remaining at values lower than that during anesthesia until the end of the experiment (Figure 2, top). In control dogs, during anesthesia the plasma β-endorphin concentration was not significantly greater than baseline. There was a rise to significantly above baseline 0.5 hour after surgery. β-Endorphin concentration then gradually fell, reaching the lowest value 6 hours after surgery. From the third to the 48th hour, the opioid concentration in the plasma was significantly lower than that during anesthesia, although not different from baseline. No significant difference was observed between the trends of ligated and control dogs (Figure 2, top).

The dogs with severe (score 0) and those with lesser (score II) deficits did not show a significant difference in β-endorphin trends. However, opioid concentration was slightly lower in the latter group (Figure 2, bottom).

Discussion

Our observations demonstrate that 60% of the dogs showed severe hind limb neurologic impairment after infrarenal aortic ligation, a percentage lower than the 100% recorded in rabbits. This is due to the fact that spinal cord blood flow in dogs is at times predominantly supplied by the thoracic aorta. However, all ligated dogs showed neurologic impairment, proving the occurrence of spinal ischemia. The histologic investigation confirmed the high susceptibility of spinal motor neurons to ischemia, unlike the dorsal horn neurons and fibers, which are relatively refractory. The correlation between clinical and histologic observations was evident, confirming the validity of our method, which also allowed separate assessments of the effects of anesthesia, surgical stress, and nervous tissue ischemia on β-endorphin production, avoiding direct manipulation of nervous tissue.

Our results show that general anesthesia with thiopental and ketamine did not significantly affect CSF and plasma concentrations of β-endorphin, as reported by other workers in plasma with pentobarbital and ketamine anesthesia. The major result of our study, however, is the demonstration that spi-
nal ischemia is significantly associated with a persistent rise in CSF β-endorphin concentration, clearly dissociated from the changes in plasma concentration, which seemed to be unaffected by the spinal stroke. This result disagrees at least in part with the work of Faden and coworkers, who found a significant increase in plasma β-endorphin immunoreactivity in cats with cervical trauma and concomitant spinal shock. The parallel between traumatic and ischemic spinal lesions is consistent since an ischemic lesion develops around a traumatic injury. However, the sharp rise of plasma β-endorphin immunoreactivity in cervically injured cats might be related to spinal shock rather than to the developing ischemic lesion. In our dogs, we did not detect important blood pressure variations at the level of the carotid artery during 2 hours after abdominal aortic ligature, and the only significant increase of plasma β-endorphin concentration in both the control and ligated dogs occurred 0.5 hour after surgery and can be correlated with surgical stress, which is known to induce opioid overproduction.

**Figure 1.** Top: Sequential measurements of immunoreactive β-endorphin concentrations in cerebrospinal fluid before and up to 48 hours after abdominal aorta ligation in dogs (arrows). Ligated dogs, solid line; sham-operated control dogs, dashed line. Bottom: Only ligated dogs, subdivided according to hind limb neurologic impairment. Score 0, dashed line; score II, solid line. Dogs with more severe impairment show high β-endorphin concentrations (not significant). Vertical bars indicate least significant difference (LSD) for p=0.05. Solid bar on vertical axis indicates range of normal values obtained previously in our laboratory.

**Figure 2.** Top: Sequential measurements of β-endorphin concentrations in plasma before and up to 48 hours after abdominal aorta ligation in dogs (arrows). Ligated dogs, solid line; sham-operated control dogs, dashed line. Bottom: Only ligated dogs, subdivided according to hind limb neurologic impairment. Score 0, dashed line; score II, solid line. No significant difference between subgroups. Vertical bars indicate least significant difference (LSD) for p=0.05. Solid bar on vertical axis indicates range of normal values obtained previously in our laboratory.
Such different behavior of the opioid in CSF and plasma may be explained by the hypothesis that plasma β-endorphin originates from the hypophysis in response to acute stress. In other words, it behaves like adrenocorticotropic, with which it shares a regulatory mechanism. CSF β-endorphin originates instead from nervous structures and changes in its concentration could be related to pathophysiologic events in nervous tissue.

It seems likely that the significant and persisting increase in β-endorphin concentration in the CSF in spinally injured dogs represents a neuronal response to ischemia since it is not present in sham-operated dogs. Confirmatory evidence could be represented by the behavior of other neurotransmitters specifically involved in ischemia, which we did not measure. Besides the clear limitation of inferring changes in the nervous system neurotransmitters or neuromodulators from measuring their concentrations in the CSF, it appears likely that the increase in the CSF opioid concentration could reflect the rise of β-endorphin content in the spinal ischemic focus. Accordingly, Hosobuchi et al documented that focal elevation of β-endorphin concentration occurred in the region of cerebral ischemia and that the absolute concentration of the opioid appeared to be correlated with the severity of the infarct. In our ligated dogs we did not detect a significant difference in CSF β-endorphin concentration between dogs with severe and dogs with moderate clinical deficits; neither was there a correlation with histologic damage. Therefore, a cause-and-effect relation between the rise in β-endorphin concentration and the degree of histologic damage cannot be established. However, in both subgroups, the increase of the opioid concentration in CSF was significantly different from that in controls, indicating that the endorphin response might be related to ischemic nervous injury. Moreover, inspection of Figure 1, bottom, clearly shows that the changes in β-endorphin concentration with time in severely injured dogs run parallel to and always exceed the values observed in moderately injured dogs.

The mechanism of action through which opiates influence neurologic function under the conditions of ischemia of nervous tissue is unknown; however, the following points should be considered: 1) increment of morphine administered intravenously progressively decrease cerebral oxygen consumption in dogs; 2) endorphin intravenicularly injected depresses motors functions, inducing catatonic behavior in rats; 3) opiates depress the firing rate of single neurons by hyperpolarizing the cell membrane; and 4) β-endorphins depress acetylcholine turnover in the rat central nervous system.

It is, therefore, likely that the elevation of endorphin concentration exacerbates neurologic deficits by further deranging, within the ischemic area, the function of neurons already altered by the lack of oxygen. In this respect it is reasonable to expect that naloxone minimizes (at least temporarily) the neurologic deficits due to ischemia since by antagonizing almost all the above-mentioned effects naloxone can improve the function of affected neurons for 20–30 minutes, its pharmacologic half-life.

References

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