environment and brought to the United States (50 percent) (*6*). The two cases in which serum samples were incorrectly judged nonteratogenic may be explained by a disorder, such as an anatomical defect in a reproductive organ (*7*), that would not be expected to find expression in maternal serum.

Thus, rat embryos can be cultured successfully on monkey serum, and changes in the serum which might be expected to occur in relation to the menstrual cycle do not appear to adversely affect the serum's ability to support embryo growth and development. Furthermore, through evaluations of serum teratogenicity it was possible to correctly identify two high-risk breeders in a group of 18 rhesus monkeys, and 12 of 14 high-risk breeders in a group of 26 pig-tailed monkeys. To our knowledge this is the first observation of a relation between the biological effects of serum and the reproductive histories of the serum donors. These results would be expected if such factors as endocrine dysfunction, immunological incompatibility, nutritional deficiencies, and chronic infectious agents—implicated in human fetal wastage (*7*)—cause comparable problems in monkeys. Nevertheless, rat embryo cultures may be particularly suited for the detection of either serum factors that inhibit embryonic development or deficiencies of essential nutrients because they can grow and develop on high concentrations of serum (90 percent by volume or higher) and because they can be cultured during the period of rapid organogenesis, when development is particularly sensitive to interference.

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## Reversal of Induced Ischemic Neurologic Deficit in Gerbils by the Opiate Antagonist Naloxone

Abstract. *Microsurgical unilateral occlusion of the right common carotid artery in 140 adult male gerbils produced homolateral cerebral ischemia and a neurologic deficit (stroke) in 42 percent (group A); the other 58 percent did not develop signs of stroke (group B). Intraperitoneal injection of the opiate antagonist naloxone (1 milligram per kilogram of body weight) reversed the signs of stroke within 3 to 5 minutes in ten out of ten group A gerbils; the effect lasted up to 30 minutes, after which stroke returned. Repeated injections of naloxone reversed stroke, but all ten gerbils died within 48 hours of ligation. However, nine other group A gerbils implanted with 10-milligram naloxone pellets had continuous reversal of signs of stroke, and four survived for more than 2 weeks. Twenty-one out of 24 group B gerbils injected intraperitoneally with morphine sulfate (5 to 30 milligrams per kilogram) 9 hours after ligation developed stroke within 3 to 20 minutes; morphine-induced stroke lasted 4 to 24 hours and could be reversed by intraperitoneal injection of naloxone. Ten out of 11 other group B gerbils injected intraperitoneally with the stereoisomeric opiate agonist levorphanol 9 hours after ligation developed signs of mild stroke that were reversed by intraperitoneal injection of naloxone. Ten other group B gerbils injected intraperitoneally with dextrorphan, the inactive enantiomer of levorphanol, 9 hours after ligation did not develop signs of stroke. Intraperitoneal injection of an enkephalin analog (Sandoz FK33824; 15 milligrams per kilogram) 9 hours after ligation did not produce stroke in ten other group B gerbils. These findings suggest the involvement of endorphins and opiate receptors in the pathophysiology of stroke and suggest the possible clinical use of opiate antagonists in humans in the acute phase of stroke.*

Recently, Brandt *et al.* (*1*) reported a hyperendorphin syndrome in a child with necrotizing encephalomyelopathy. After a protracted clinical course, the child lapsed into coma; intravenous administration of less than 1 mg of naloxone reversed his coma in four out of seven trials. High concentrations of endorphins were found in cerebrospinal fluid samples collected during the course of therapy and in the postmortem brain. As part of an ongoing experimental protocol designed to study the effects of neuropeptides on the function of the central nervous system, we recently found that hemiplegia secondary to cerebral ischemia in two patients could be reversed totally for up to 20 minutes by intravenous injection of 0.4 mg of naloxone (*1a*). Injection of placebo saline had no effect. Significantly, reversal of hemiplegia was not accompanied by any change in vital signs. Hemiplegia in both patients could be reversed repeatedly by intravenous injection of naloxone; in one patient, reversal of hemiplegia was obtained by naloxone injection over the course of several months. Moreover, in one of these patients, after the hemiplegia had resolved spontaneously, intravenous injection of morphine sulfate produced hemiplegia that was immediately reversed by intravenous injection of naloxone (0.4 mg). The hemiplegic condition of a third patient who had radiographically documented areas of cerebral infarction could not be reversed by intravenous injection of naloxone.

Because of this remarkable reversal of hemiplegia by naloxone, we studied the effects of naloxone on surgically induced ischemic neurologic deficit in gerbils that developed hemiparesis after occlusion of the right common carotid artery. We compared the opiate receptor binding capacity between the ischemic hemispheres and the contralateral hemisphere in these animals. We also studied the induction of stroke caused by the injection of morphine sulfate and the effects of stereoisomeric opiate agonists in gerbils that had not developed hemiplegia after occlusion of the common carotid artery.


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For the past 10 years, gerbils have been used as a laboratory model to study the pathophysiology of cerebral ischemia and ischemic cerebral edema (*2*). Gerbils share with humans the propensity to develop cerebral infarction and stroke after occlusion of one carotid artery (*2*, *3*). The anatomic structure equivalent to the Circle of Willis in humans is incomplete in gerbils; gerbils have no posterior communicating artery, and 30 to 50 percent of gerbils either have no anterior communicating artery or have an artery of such small caliber that only low blood flow can be shunted to either hemisphere if the common carotid artery is occluded. Thus 30 to 50 percent of adult gerbils develop hemiplegia (stroke) secondary to cerebral ischemia after unilateral occlusion of the common carotid artery (*2*–*4*).

We anesthetized 140 adult male gerbils, weighing 80 to 100 g each, with 40 mg/kg (intraperitoneal) of pentobarbital. Using an operating microscope, we isolated the right common carotid artery, coagulated it with microbipolar forceps, and divided it with scissors. Then the wound was closed, and the gerbils were returned to their cages. They recovered from anesthesia within 2 hours and were alert and responsive. Fifty-nine of 140 gerbils (42 percent) began to manifest signs of stroke 2 to 4 hours after surgery (group A); none developed stroke more than 4 hours after surgery. The most frequently seen neurologic deficit in group A gerbils was hemiparesis that was manifested by a paucity of movement on one side of the body, a splayed-out hindlimb during ambulation, and failure to resist a drag test, which is a measure of the ability of the gerbil to resist a forced lateral pulsion. Some group A gerbils that did not develop hemiparesis as defined above exhibited a circling behavior, usually to the right. The other 81 gerbils that were operated on did not develop signs of stroke (group B).

Table 1. Stroke induced by the administration of opiate agonists to gerbils with carotid ligation that did not develop postsurgical stroke (group B).

| Drug administered | Dose (mg/kg) | Number that developed stroke/ number injected |
|---|---|---|
| Morphine | 5 to 30 | 21/24 (two died) |
| Dextrorphan | 3 | 0/5 |
| Dextrorphan | 15 | 0/5 |
| Levorphanol | 5 | 6/7 |
| Levorphanol | 15 | 4/4 |
| Sandoz compound FK33824 | 15 | 0/10 |

Fifteen group A gerbils that received no treatment after surgery were used as controls. After the development of hemiparesis, they progressively became less responsive, then obtunded, and finally lapsed into coma. Saline (1 ml) injected intraperitoneally 9 hours after ligation (*5*) did not change their neurologic status. Nine died within 12 hours, four within 24 hours, and two within 48 hours of surgery (Fig. 1). Postmortem examination of their brains showed varying amounts of edema and infarction in the right cerebral hemisphere; these findings are in agreement with the findings of others (*2*–*4*).

Naloxone (1 mg/kg) was injected intraperitoneally into ten other group A gerbils. Hemiparesis or the circling behavior or both disappeared within 3 to 10 minutes (mean, 5.4 minutes) in all ten gerbils, and they responded equally well in both directions to the drag test. Naloxone reversal of hemiparesis lasted 20 to 30 minutes, after which the deficit returned. Repeated injections of naloxone consistently reversed all neurologic deficit. However, all ten gerbils were dead within 48 hours.

Therefore, 10-mg pellets of naloxone were implanted subcutaneously in nine other group A gerbils. Two survived 24 hours, three survived 36 hours, but four survived deficit-free for more than 2 weeks (Fig. 1). Gerbils that died appeared to be well until a few hours before death, when they rapidly became obtunded and comatose. Postmortem examination of their brains showed marked right cerebral edema. The four gerbils that survived more than 2 weeks had no residual neurologic deficit. Postmortem examination of their brains revealed that one had definite encephalomalacia and atrophy in the right occipital area.

Cerebral ischemia caused by occlusion of the carotid artery in group B gerbils was not severe enough to cause hemiplegia or other signs of stroke. Remarkably, however, intraperitoneal injection of morphine sulfate (5 to 30 mg/kg; median dose, 15 mg/kg) within 3 to 20 minutes (mean, 8 minutes) induced a left hemiparesis that lasted 4 to 24 hours (mean, 18 hours) in 21 out of 24 group B gerbils (Table 1). Two gerbils died. This morphine-induced stroke was reversed within 5 minutes in ten out of ten gerbils by intraperitoneal injection of naloxone (1 mg/kg). Because the long-term protective effect of naloxone administered by the subcutaneously implanted pellet was documented in group A gerbils, the procedure was not repeated in gerbils with morphine-induced stroke.

The stereoisomeric opiate agonist levorphanol was injected intraperitoneally into 11 other group B gerbils 9 hours after ligation; ten developed signs of mild stroke that were reversed by naloxone (1 mg/kg) (Table 1). Dextrorphan, the inactive enantiomer of levorphanol, was injected intraperitoneally into ten other group B gerbils; none developed stroke (Table 1). Thus with these agonists, stroke induction is a stereospecific phenomenon. Similarly, an enkephalin analog (Sandoz FK33824; 15 mg/kg) was injected intraperitoneally into ten group B gerbils at a dose that is 80 times the mouse $ED_{50}$ (median effective) analgesic dose. None developed stroke, although all were totally analgesic for 24 hours (Table 1).

We compared the opiate receptor binding capacity between the ischemic

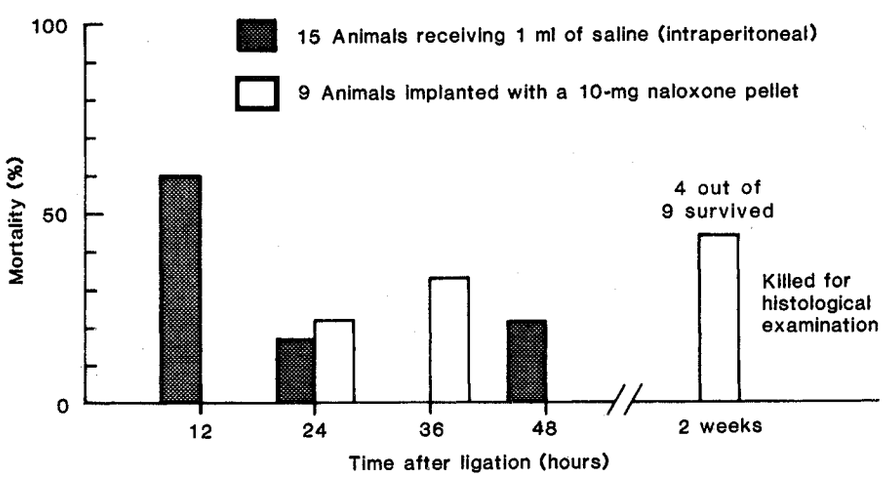


Fig. 1. Time of death in 24 gerbils with unilateral carotid ligation that developed postsurgical stroke (group A).

and nonischemic hemisphere by using [$^3$H]naloxone and [$^3$H]dihydromorphine in untreated group A gerbils (*6*). Twenty gerbils were killed 9 hours after ligation, and the hemispheres were dissected free of the cerebellum and the brain stem and then separated. Crude synaptosomal fractions were isolated from each hemisphere, and a standard binding assay similar to that reported by Pert and Snyder (*7*) was performed. Scatchard analysis of the data indicates that there was no significant difference in either the capacity or affinity of binding to both high- and low-affinity sites between the ischemic (right) and the control (left) hemispheres.

Brains from several untreated group A gerbils were analyzed for concentrations of immunoreactive β-endorphin–like material by F. E. Bloom at the Salk Institute, La Jolla, California. Preliminary data (corrected for protein) indicate that the concentration of immunoreactive β-endorphin–like material was 40 to 80 percent higher in the ischemic right hemisphere than in the left control hemisphere.

These observations show that naloxone reverses the neurologic-deficit caused by cerebral ischemia in gerbils and that continuous administration of naloxone reduces mortality due to stroke. Our results further suggest that the action of opiates and naloxone on the neurologic deficit is stereospecific and that there appears to be no significant alteration in receptor binding capacity or the affinity of ligand binding to receptors.

Recently, Faden *et al.* (*8*) reported that systemically administered naloxone significantly prevented the development of hypotension after spinal cord injury in cats, which in turn prevented the development of extensive ischemic damage in the face of traumatic injury. Intracerebral injections of naloxone at doses much lower than those administered intraperitoneally produced the same protective effect on spinal cord function. Faden *et al.* postulated that hypotension produced by spinal cord injury may be mediated centrally and suggested the involvement of the endorphin system.

We have not been able to measure blood pressure in gerbils, although respiratory rate and thoracic excursion did not seem to be altered by either naloxone or morphine at the doses given. In several gerbils, arterial blood samples were obtained by cardiac puncture; gerbils treated with naloxone or opiate agonists had no significant alterations in arterial partial pressure of $O_2$, partial pressure of $CO_2$, or *p*H compared to untreated gerbils. In the absence of other causes, cerebral ischemia often induces systemic hypertension in animals or humans (*9*). Moreover, there were no changes in vital signs in the two patients in whom hemiplegia was reversed by naloxone (*1a*). Therefore, we do not feel that our observation is related to the pathophysiology that led to the observation of Faden *et al.*

The depressive effect of intracerebral administration of β-endorphin on motor activity is well known (*10*). On the basis of our observations in gerbils and our findings in humans, it is tempting to assume that cerebral ischemia causes an increase in the level of β-endorphin that in turn, by an unknown mechanism, produces hemiparesis. If there is no cerebral infarction, this effect can be reversed by naloxone. Our finding that naloxone did not reverse hemiplegia in a patient with focal cerebral infarction supports this hypothesis (*1a*). However, if the ischemic insult is insufficient to produce motor manifestations, administration of exogenous opiate may be sufficient to alter homeostasis and produce stroke. As shown by our results in gerbils, stroke induced by opiate agonists is a stereospecific phenomenon. The enkephalin analog Sandoz FK33824, which has potent analgesic activity in rodents (*11*), did not produce signs of stroke in group B gerbils. These observations are novel and suggest that treatment with naloxone may significantly improve functional neurologic recovery and reduce mortality after ischemic cerebral insult in humans.

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## Epinephrine Reduction of Heme: Implication for Understanding the Transmission of an Agonist Stimulus

Abstract. *Alpha-adrenergic agonists that promote platelet aggregation were found to reduce ferric heme to ferrous heme. Agents that bind iron in heme inhibited epinephrine-induced platelet aggregation. It is proposed that epinephrine first binds to its receptor and then reduces an adjacent heme group to transmit its agonist stimulus.*

The mechanism of transduction of receptor-mediated signals across biological membranes is of considerable interest (*1*). Although the platelet α-adrenergic binding site has been studied (*2*), the chemical or physical changes that transmit the agonist signal are not known. We found that epinephrine and norepinephrine can reduce ferric ($Fe^{3+}$) heme to ferrous ($Fe^{2+}$) heme (*3*). We now present evidence to suggest that epinephrine causes platelet aggregation through its ability to reduce heme.

Figure 1 shows heme reduction (*4*) by several compounds with known interactions with the platelet α-adrenergic receptor. The rank order of heme reduction (epinephrine > norepinephrine > epinine > dopamine > phenylephrine) was similar to the rank order of the same compounds as agonists (*5*). Phentolamine, which binds to the platelet receptor with high affinity (*2*) but is an inhibitor, not an agonist, was ineffective at reducing heme. Phenylephrine, which binds tightly to the platelet receptor and is an inhibitor of epinephrine aggregation but has weak agonist activity itself (*6*), caused slight but significant heme reduction at high concentrations. All com-


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