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Failure of MIF-1 or Naloxone to Reverse Ischemic-Induced Neurologic Deficits in Gerbils

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KASTIN, A. J., C. NISSEN AND R. D. OLSON. *Failure of naloxone or MIF-1 to reverse ischemic-induced neurologic deficits in gerbils.* PHARMAC. BIOCHEM. BEHAV. 17(5) 1083–1085, 1982.—MIF-1 and naloxone exert similar actions in several situations. Since naloxone, at a dose of 1 mg/kg IP, has been reported to reverse the neurologic deficits of gerbils whose right common carotid artery had been occluded, MIF-1 was tested under the same conditions and the effects compared with naloxone. Doses of 0.1, 1.0, and 10.0 mg/kg IP of MIF-1 and naloxone did not significantly alter the signs of either moderate or severe neurologic deficits. Thus, the results of this study with gerbils do not add evidence for the use of these opiate antagonists in strokes.

Vascular stroke Opiate antagonist MIF-1 Naloxone

SEVERAL experimental conditions have been found in which Pro-Leu-Gly-NH_2 (MIF-1) acts acutely like naloxone. These include antagonism of analgesia in the tail-flick test, as well as the hypothermia and hypomotility induced by morphine or opiate peptides [5, 6, 17]. Somewhat similar dose-response relationships of naloxone and MIF-1 have been observed on temperature and deprivation-induced drinking [11, 12, 17]. The more chronic effects of MIF-1 on opiate tolerance remain controversial [1, 8, 14–16].

Unlike naloxone, however, a moderate dose of MIF-1 does not decrease food intake in VMH-lesioned rats [6,7] nor does it inhibit the effects of morphine on the Straub tail phenomenon, withdrawal jumping, or vas deferens [5,6]. The effects of MIF-1 on morphine-induced contractures of the guinea pig ileum are also probably insufficient to explain its actions as an opiate antagonist [2,5]. Direct measurement of striatal mu and delta receptors, moreover, failed to reveal any competition for these sites by MIF-1 [10].

The report [3] of reversal of induced ischemic neurologic deficits in gerbils by the opiate antagonist naloxone provided another situation in which to test the possible similarity of action of naloxone and MIF-1. The gerbil lacks connecting arteries between the basilar and carotid systems [4,9]. Microsurgical unilateral occlusion of the right common carotid artery in adult male gerbils was reported to produce homolateral cerebral ischemia and a neurologic deficit (stroke) that was reversed in 10 out of 10 animals within a few minutes after IP injection of naloxone at a dose of 1 mg/kg [3]. We used the same dose of naloxone and MIF-1 they used in addition to doses 10 times higher and 10 times lower, in the same procedure, to evaluate the effects of these opiate antagonists.

METHOD

As described elsewhere [3], about 200 male Mongolian gerbils, weighing about 80 g, were obtained from Tumblebrook Farms (West Brookfield, MA) and housed for one week under conditions of 12 hr light:12 hr dark. After anesthesia with pentobarbital (40 mg/kg IP), the right common carotid artery was isolated, coagulated with microbipolar forceps, and severed. The wound was closed with clamps and the gerbils were allowed to recover for 4 hours. During this time about 40% of the animals showed signs of neurologic deficit and were used for the experiment. Nine signs were scored as present or absent for each animal 5 min before injection of the test substance as well as 5, 10, and 25 min later. These were ptosis, circling, splayed leg, rolling, darting, lying on the back, a hunched posture characterized by decreased activity, torticollis, and clonic spasms of the hind paws. Those gerbils in which behaviors of circling, rolling, and lying on the back were particularly prominent were classified as severe and analyzed separately from as well as together with the remainder of the animals classified as having moderate deficits.

In a balanced design, each group of gerbils received a single injection at time 0 of MIF-1, naloxone, or diluent (0.9% NaCl made to 0.1 M with acetic acid). All solutions were coded by someone other than the observer and consisted of diluent or 0.1, 1.0, or 10.0 mg/kg of the naloxone or MIF-1. Each dose was injected IP into 6 gerbils with severe neurologic deficits and into 6 gerbils with moderate neurologic deficits.

The largest difference between the number of signs exhibited by a gerbil before injection of the test material and at

TABLE 1

MEAN NUMBER OF SIGNS OF NEUROLOGIC DEFICITS CHANGED BY INJECTION OF MIF-1, NALOXONE, OR DILUENT

		MIF-1 (mg/kg)			Naloxone (mg/kg)		
Neurologic Deficit	Diluent	10	1	0.1	10	1	0.1
Severe	−1.8	−1.0	−1.0	−1.8	−0.8	−2.0	−0.2
Moderate	−0.3	−0.5	+0.2	+0.5	−0.7	−0.2	−0.2

(−)=Reversal and (+)=addition of any of the 9 signs.
n=6 in each group.

TABLE 2

MEAN GLOBAL SCORE OF CHANGE IN NEUROLOGIC DEFICITS AFTER MIF-1, NALOXONE, OR DILUENT

		MIF-1 (mg/kg)			Naloxone (mg/kg)		
Neurologic Deficit	Diluent	10	1	0.1	10	1	0.1
Severe	0	0	0	+0.3	0	0	+0.3
Moderate	+0.2	+0.2	−0.2	+0.3	0	0	+0.5

Each rat was scored at −1=improved, +1=worse, or 0=same.
n=6 in each group.

any of the subsequent times during the next 30 min of observation was used for the statistical analysis. In addition, a global impression was scored as improved (−1), no change (0), or worse (+1) by the observer who had injected the coded solutions.

For each set of results, differences between the gerbils with signs of severe neurologic deficits were compared to those with signs of moderate neurologic deficits by the Kolmogorov-Smirnov two-sample test [13]. Differences for the global observations as well as the number of changed signs among the 7 independent groups (3 doses of MIF-1, 3 doses of naloxone, and 1 dose of diluent) were compared by the Kruskal-Wallis one-way analysis of variance [13].

RESULTS

The number of signs of neurologic deficits (Table 1) was not reliably changed by any of the treatments (H=3.14, a value of 12.59 being needed for statistical significance at the 5% level). There was a general tendency for gerbils with severe neurologic deficits to show a greater reduction in the number of signs of neurologic deficits over the 30 min test period as compared with the changes shown by animals with moderate neurologic deficits, but the obtained value of D by the Kolmogorov-Smirnov test did not exceed the critical value necessary for statistical significance.

The global impression of change in the neurologic deficits of the gerbils (Table 2) did not differ among any of the 7 groups to a statistically significant extent (H=5.45). Similarly, there was no reliable difference in overall change between gerbils with severe or moderate signs of neurologic deficits.

DISCUSSION

Neither MIF-1 nor naloxone reliably reversed the neurologic deficits induced in gerbils by unilateral occlusion of the common carotid artery. This is in contrast to the report appearing earlier this year [3], in which naloxone reversed the ischemia-induced deficits. The percent of operated animals manifesting neurologic deficits was the same in both investigations as were essentially all procedural details.

It is possible, however, that some important factor differed between the two studies. The large variability in each group of our gerbils, even those receiving diluent, also raises the possibility that a small functional improvement might have been missed. It was clear, nevertheless, that no marked reversal of neurologic signs occurred. Only 17% of gerbils receiving 1 mg/kg naloxone showed any overall improvement in contrast to 100% in the previous study [3].

Our failure to significantly reverse the signs resembling stroke in gerbils by either MIF-1 or naloxone does not exclude the possibility that other opiate antagonists, perhaps even naturally occurring peptides, may be active in this system. It also does not preclude the possibility that some opiate antagonists may be found to be effective in other neurologic disorders.

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