Effects of 4-(*o*-Benzylphenoxy)-*N*-Methylbutylamine Hydrochloride (MCI-2016) on Survival Time and Brain Monoamine Levels in Bilaterally Carotid-Artery-Ligated Gerbils

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It is well-known that bilateral occlusion of the carotid arteries produces cerebral ischemia in the Mongolian gerbil (1, 2). Ligation of one artery was used to produce a severe neurological deficit and a high mortality, which resembled the syndrome of middle cerebral arterial occlusion in humans (3). Bilateral carotid ligation in the gerbil is uniformly fatal because the collateral blood supply from the vertebral-basilar system is insufficient to prevent total cerebral infarction when the carotid flow is blocked (4).

MCI-2016 is a new drug which has anti-anoxic, EEG activating and memory retrieval effects in laboratory evaluations (5–7). The drug also stimulates the central noradrenergic mechanism (8). In addition to these pharmacological properties, MCI-2016 has been reported to be effective for the treatment of cerebrovascular disease in recent clinical trials (9).

In the present study, we examined the effects of MCI-2016 on cerebral ischemia produced by bilateral carotid ligation in the gerbil.

Male Mongolian gerbils (60–108 g) were rapidly operated on under light ether anesthesia. Bilateral carotid arteries were ligated with great caution to avoid vagal irritation or injury which might cause hypoxia due to respiratory disturbances. After ligation, two studies which consisted of examination of survival time and assays of brain monoamine levels were performed independently.

In order to determine the survival time, the behavior of animals was observed in a transparent plastic box (30×30×30 cm) until 36 hr after ligation.

In another experiment, animals were sacrificed 1 hr after occlusion, and the brain was removed quickly. Norepinephrine (NE) and dopamine (DA) levels in whole brain excluding the cerebellum were determined by the slightly modified method of Feinhard et al. (10), and serotonin (5-HT) levels were determined by the method of Mefford et al. (11) by means of high-performance liquid chromatographic techniques.

Drugs dissolved in saline were administered intraperitoneally 30 min before ligation.

1. Changes in survival time: After recovery from anesthesia, all gerbils (N=71) showed the stroke syndrome which consisted of hair rough-up, splayed limb position, circling and convulsions, and died within 36 hr.

As shown in Table 1, the mean survival time in the control (saline) group was 2.4 ± 0.5 hr. Although the mean survival time was slightly prolonged by pretreatment with 10 mg/kg MCI-2016, the drug at 25 mg/kg was more effective; four animals given 25 mg/kg of MCI-2016 survived over 12 hr.

Ca-hopantenate, a cerebral metabolic activator, also showed a tendency to prolong the survival time.

2. Changes in brain monoamine levels: The levels of NE, DA and 5-HT in the ligated group decreased to 68, 82 and 67% of those of the sham operated group, respectively (Table 2). The reductions of monoamine contents are a well-known characteristic of cerebral ischemia that produces neurological deficits (12, 13).

MCI-2016 at 10 mg/kg induced slight inhibition of decreases in NE levels, while the drug failed to show significant antago-

Table 1. Effects of MCI-2016 and Ca-hopantenate on survival time in bilaterally carotid-ligated gerbils

Treatment	Dose (mg/kg, i.p.)	N	Survival time (hr) (mean±S.E.M.)	No. of survival animals over 12 hr
Saline	—	20	2.4±0.5	0
MCI-2016	10	17	2.7±1.0	1
	25	17	8.1±2.7*	4
Ca-hopantenate	100	17	4.0±2.0	1

*P<0.05 vs. saline control.

Table 2. Effects of MCI-2016 on norepinephrine (NE), dopamine (DA) and serotonin (5-HT) levels in the brain of bilaterally carotid-ligated gerbil

Treatment	Dose (mg/kg, i.p.)	N	Contents (ng/g) ^a		
			NE	DA	5-HT
Sham operation	—	9	363±11	1151±15	334±11
Ligation:					
Saline	—	9	246±16 ^{b**}	944±37 ^{b**}	224±10 ^{b**}
MCI-2016	10	9	266±28 ^{b**}	920±54 ^{b**}	258±10 ^{b** c*}
	25	9	312±21 ^{b* c*}	1032±35 ^{b**}	255±10 ^{b** c*}

^a Mean±S.E.M. Statistical significance: ^b vs. sham operation, ^c vs. saline ligation, *P<0.05, **P<0.01.

nism to reduction of 5-HT levels. Also, 25 mg/kg of MCI-2016 significantly attenuated the reduction of NE and 5-HT contents by occlusion.

Global cerebral ischemia by bilateral occlusion of carotid arteries in the gerbil does not so closely resemble cerebrovascular diseases in humans as does the unilateral ligation (4). However, it is technically easy for the study of cerebral ischemia per se. Since this method resulted in 100% mortality, judgement of the drug effect is clear and simple, i.e., survival vs. death of the animals used.

The present study revealed the stroke syndrome in all animals examined and reductions of monoamine contents, especially NE and 5-HT contents, after bilateral carotid ligation. This evidence was similar to many previous reports (1, 2, 14). Some parts of the neurological deficits will be provoked by these monoamines (15).

MCI-2016 prolonged survival time and inhibited dose-dependently the reduction of NE and 5-HT contents. We reported that MCI-2016 increases NE and 5-HT levels in normal rats in neurochemical evaluations (8). So the ameliorating effects of MCI-2016 in

this gerbil model may be due to these neurochemical properties of MCI-2016. However, it is doubtful whether MCI-2016 acts on monoamine systems similarly as it does in normal conditions under this acute and severe ischemia.

There is other evidence that MCI-2016 inhibits the release of NE only by high K⁺ stimulation in the neuroblastoma system in vitro (M. Mitsuka et al., unpublished data). This condition of high K⁺ stimulation might resemble more closely excess excitation of neurones induced by bilateral carotid ligation. So it is suggested that the inhibition of NE release by MCI-2016 would partly be involved in the amelioration of survival time induced by intense cerebral ischemia.

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