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Comparison of the effects of bifemelane hydrochloride, idebenone and indeloxazine hydrochloride on ischemia-induced changes in brain monoamines and their metabolites in gerbils

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Summary. Bifemelane hydrochloride (bifemelane), idebenone and indeloxazine hydrochloride (indeloxazine) are used clinically to reduce apathy and other emotional disturbances in patients with cerebrovascular disease. In gerbil brains, ischemia affects many monoaminergic neurotransmitters and their metabolites. In the present study, the effects of treatment with bifemelane, idebenone and indeloxazine on ischemia-induced changes in monoamines and their metabolites were studied in ischemic gerbil brains. Although these drugs had no effect on the monoaminergic neurotransmitters or their metabolites in sham-operated animals, in the ischemic brains both dopamine and serotonin turnovers were abnormal after idebenone or indeloxazine treatment. Bifemelane, in contrast, tended to correct the ischemia-induced changes in the dopaminergic and serotonergic systems in the cerebral cortex, hippocampus and thalamus + midbrain. From the present results and those in previous reports, we conclude that bifemelane is more appropriate than idebenone or indeloxazine as a treatment for the ischemia-induced changes in monoaminergic neurotransmitter systems.

Keywords: Bifemelane hydrochloride, idebenone, indeloxazine hydrochloride, ischemic gerbils, monoamines and their metabolites.

Introduction

The Mongolian gerbil has been used as a model of cerebral ischemia and of some forms of human stroke (Kahn, 1972). Cerebral ischemia in gerbils results in a marked and rapid change in neurotransmitters and their metabolites in the brain (Levy and Duffy, 1975; Lust et al.,1975; Kobayashi et al., 1977; Cvejic

et al., 1980; Harrison and Ellam, 1981; Egawa et al., 1984; Ogawa et al., 1988 b; Haba et al., 1990). These changes in neurotransmitters in the ischemic brain may have secondary deleterious effects on cerebral blood flow and metabolism (Wurtman and Zervas, 1974; Harrison and Ellam, 1981). It is likely that many of the neurological and psychological symptoms of cerebrovascular disease, cognitive and memory disturbances, reduced spontaneity, emotional disturbance, headache and dizziness would be alleviated by inhibition of ischemia-induced changes in these neurotransmitters.

Cerebral vasodilators and metabolic enhancer have been reported to have antianoxic effects (Rossignol and Margaret, 1980; Hoffmeister et al., 1982). They have been demonstrated in animal experiments to be effective for treating cerebral disturbances caused by ischemia (Ogawa et al., 1988 b, 1989; Wurtman and Zervas, 1974). Recently, some of these drugs, including bifemelane hydrochloride (bifemelane), idebenone and indeloxazine hydrochloride (indeloxazine) have been used to treat patients with cerebrovascular diseases. Bifemelane, 4-(*o*-benzylphenoxy)-N-methylbutylamine hydrochloride, has been proved, both experimentally and clinically, to be able to reduce apathy and other emotional disturbances, such as amnesia and memory loss, associated with cerebrovascular disease, aging and senile dementia (Ogawa et al., 1988 a, b; Miyazaki et al., 1989; Nagai et al., 1990; Yamamoto et al., 1985; Ohara et al., 1989; Itakura et al., 1988). Idebenone, 6-(10-hydroxydecyl)-2, 3-dimethoxy-5-methyl-1, 4-benzoquinone, improved the amnesitic performance deficits induced by the cerebral ischemia (Yamazaki et al., 1984) and attenuated the decrease in acetylcholine (ACh) concentration in rat brain exposed to a very short duration of ischemia (Kakihana and Yamazaki, 1984). Indeloxazine, 2-(7-indenyloxy-methyl) morpholine hydrochloride, possess antianoxic effects (Yamamoto and Shimizu, 1987 a), antireserpine actions without anticholinergic activity (Tachikawa et al., 1979) and facilitory effects on learning behavior (Yamamoto and Shimizu, 1987 b).

The present study compared the effects of these three drugs on monoamines and their metabolites in four brain regions (cerebral cortex, hippocampus, striatum and thalamus + midbrain) in ischemic gerbils.

Materials and methods

Preparations of cerebral-ischemic gerbils

Gerbils weighing 70–90 g were used. Under ketamine anesthesia (100 mg/kg body weight, i.p.), silk threads were placed around both common carotid arteries without interrupting the carotid blood flow. On the next day, cerebral ischemia was induced via ligation of both common carotid arteries under light ether anesthesia. Ischemia was maintained for 15 min and then the animals were killed with microwave irradiation (4.8 kW, 1.1 sec.) (Ogawa et al., 1988 b). Sham-operated gerbils were treated in the same way, but without occlusion. After microwave irradiation, the cerebral cortex, hippocampus, striatum and thalamus + midbrain were dissected on an ice-plate according to the method of Glowinski and Iversen (1966), and were stored at − 80 °C until analysis.

Drug treatments

Bifemelane (15 or 30 mg/kg), idebenone (10 or 30 mg/kg) or indeloxazine (10 or 30 mg/kg) were injected intraperitoneally 30 min prior to the ligation. Bifemelane and indeloxazine were dissolved in physiological saline, and idebenone was suspended in 5% gum arabic.

Sample preparation

The brain tissues were rapidly homogenized in ice-cold 0.2 M perchloric acid containing EDTA (0.01%). The homogenate was frozen and thawed before centrifugation at 10,000 g for 15 min. The supernatant was filtered through a Millipore Type HV 0.45 μm membrane filter. Ten μl of the aqueous layer was injected directly into a high performance liquid chromatograph (HPLC) for monoamine determination (Haba et al., 1990).

Monoamines and their metabolites

Regional concentrations of monoamines and their metabolites in the brain were determined using HPLC with electrochemical detection as previously reported (Lastey et al., 1984) with minor modifications (Haba et al., 1990). The HPLC system consisted of a delivery pump (Model L-4000 W, Yanagimoto, Kyoto, Japan), and an analytical column (LiChrospher RP-18, 250 mm × 4 mm internal diameter Cica-Merck) protected by a LiChrisorb RP-18 guard column (4 mm × 4 mm internal diameter Cica-Merck). An electrochemical detector (Model VMD-501, Yanagimoto) with glassy carbon was used at a voltage setting at 0.75 V versus an Ag/AgCl reference electrode. The mobile phase consisted of 100 mM potassium phosphate buffer (pH 3.1) containing 13% acetonitrile, 770 mg/l sodium octanesulfonate and EDTA, at a flow rate of 0.8 ml/min.

Protein concentration

Protein concentration was determined with a Bio-Rad protein assay kit with bovine serum albumin as a standard.

Statistical analysis

Statistical analysis was performed by one-way ANOVA followed by Mann-Whitney U-test with $p < 0.05$ taken as significant.

Results

Dopamine (DA) and its metabolites concentrations

There were no differences in the concentrations of DA or its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in any region of the brain in the sham-operated group, between the vehicle-treated and drug-treated animals, indicating that none of the drugs affected dopaminergic system in normal animals (Table 1).

The effects of bifemelane, idebenone and indeloxazine on ischemia-induced changes in DA and its metabolites are also shown in Table 1. Although the DA level in the striatum was markedly lower in ischemic gerbils, it was higher in the thalamus + midbrain. The cerebral cortical DOPAC level was higher than that in the sham-operated group, but striatal DOPAC level was depressed. Furthermore, bilateral ischemia resulted in higher HVA concentrations in all

Table 1. Effects of bifemelane, idebenone and indeloxazine on ischemia-induced changes in DA and its metabolites (ng/mg protein) in four brain regions

	Sham-operated group				Ischemic group						
	vehicle	bifemelane (30 mg/kg)	idebenone (30 mg/kg)	indeloxazine (30 mg/kg)	vehicle	bifemelane		idebenone		indeloxazine	
						(15 mg/kg)	(30 mg/kg)	(10 mg/kg)	(30 mg/kg)	(10 mg/kg)	(30 mg/kg)
Cerebral cortex											
DA	6.46 ± 1.18	8.58 ± 0.88	7.47 ± 0.74	5.47 ± 0.97	7.15 ± 0.74	5.91 ± 1.04	6.59 ± 0.27	3.97 ± 0.93	3.12 ± 1.26	6.35 ± 0.78	5.6 ± 1.30
DOPAC	0.46 ± 0.04	0.61 ± 0.07	0.57 ± 0.07	0.67 ± 0.07	0.62 ± 0.08*	0.50 ± 0.15	0.71 ± 0.05**	0.43 ± 0.08	0.30 ± 0.05*	0.59 ± 0.08	0.41 ± 0.06
HVA	2.28 ± 0.20	2.29 ± 0.29	1.76 ± 0.19	1.80 ± 0.19	2.08 ± 0.21	2.11 ± 0.36	2-.99 ± 0.14**++	2.56 ± 0.58	1.55 ± 0.16	2.32 ± 0.27	2.55 ± 0.28
Hippocampus											
DA	0.88 ± 0.10	1.56 ± 0.93	1.34 ± 0.50	1.29 ± 0.30	0.82 ± 0.30	1.53 ± 0.22+	1.83 ± 0.41+	0.81 ± 0.26	1.52 ± 0.24	0.40 ± 0.11	1.62 ± 0.52
DOPAC	0.38 ± 0.03	0.34 ± 0.04	0.37 ± 0.10	0.47 ± 0.05	0.55 ± 0.17	0.56 ± 0.28	0.34 ± 0.04	0.35 ± 0.04	1.44 ± 0.26	0.36 ± 0.07	1.25 ± 0.11
HVA	0.79 ± 0.07	1.35 ± 0.61	0.70 ± 0.22	1.32 ± 0.42	2.06 ± 0.42*	1.23 ± 0.21	2.00 ± 0.32	1.42 ± 0.49	1.02 ± 0.58	1.07 ± 0.15	0.60 ± 0.08+
Striatum											
DA	72.4 ± 15.8	55.8 ± 5.31	44.8 ± 0.44	48.9 ± 3.00	14.3 ± 3.49**	9.14 ± 1.80**	14.6 ± 0.55**	14.3 ± 0.57*	15.9 ± 2.00	16.1 ± 0.53*	12.7 ± 3.22
DOPAC	5.69 ± 1.69	1.69 ± 0.36	2.67 ± 0.26	2.38 ± 0.26	1.64 ± 0.21*	5.15 ± 1.87+	8.34 ± 1.54++	0.57 ± 0.10	2.08 ± 0.70	2.73 ± 0.26+	3.53 ± 0.90
HVA	0.88 ± 0.14	0.68 ± 0.12	0.71 ± 0.07	0.64 ± 0.04	2.27 ± 0.45*	1.91 ± 0.42*	1.48 ± 0.15*	11.6 ± 1.92*+	18.3 ± 2.80*+	10.7 ± 1.12*+	12.0 ± 2.74*+
Thalamus + midbrain											
DA	0.86 ± 0.16	0.75 ± 0.05	0.77 ± 0.24	0.78 ± 0.09	1.44 ± 0.11*	1.05 ± 0.20	1.45 ± 0.39	6-.84 ± 1.67**++	4.87 ± 1.22	2-.62 ± 0.34**++	2.77 ± 0.65
DOPAC	1.30 ± 0.38	0.76 ± 0.21	0.83 ± 0.08	0.76 ± 0.17	2.11 ± 0.48	1.53 ± 0.28	1.65 ± 0.51	1.69 ± 0.20	1.30 ± 0.14		
HVA	0.31 ± 0.05	0.27 ± 0.11	0.27 ± 0.03	0.31 ± 0.04	5.55 ± 0.94**	0.54 ± 0.07*++	0-.93 ± 0.13**++	1.64 ± 0.83 4.75 ± 1.64**	4.08 ± 0.41	0.28 ± 0.03 1.28 ± 0.09**	2.26 ± 0.41

Values are means ± SEM (n = 6)
* $p < 0.05$, ** $p < 0.01$ vs. sham-operated controls
+ $p < 0.05$, ++ $p < 0.01$ vs. ischemic controls

regions except the cerebral cortex. With bifemelane treatment, the hippocampal DA level was about 200% greater than the ischemic control, the DOPAC level was dose-dependently higher in the striatum, and prevented the ischemia-induced increase in HVA level in the thalamus + midbrain. Idebenone pre-treatment markedly increased or decreased the DA levels in the thalamus + midbrain or cerebral cortex, respectively, and increased the HVA level in the striatum, comparing with ischemic control. On the other hand, indeloxazine pretreatment increased the DA level in the thalamus + midbrain and the HVA levels in both striatum and thalamus + midbrain.

In the ischemic gerbils, DA turnover [(DOPAC + HVA)/DA] was lower in hippocampus and higher in the striatum and thalamus + midbrain (Fig. 1). Cerebral cortical DA turnover tended to be decreased with ischemia. Bifemelane normalized DA turnover in all brain regions except the striatum. In idebenone-treated animals DA turnover was markedly lower in the thalamus + midbrain, and higher in other regions, when compared with sham-operated and ischemic gerbils. In contrast, normalized effects on the DA turnover was observed in hippocampus and thalamus + midbrain with high-dose indeloxazine treatment.

Noradrenaline (NA) concentration

Table 2 shows the effects of these drugs on the NA level in brains of ischemic gerbils. There were no significant differences in the NA concentrations in the

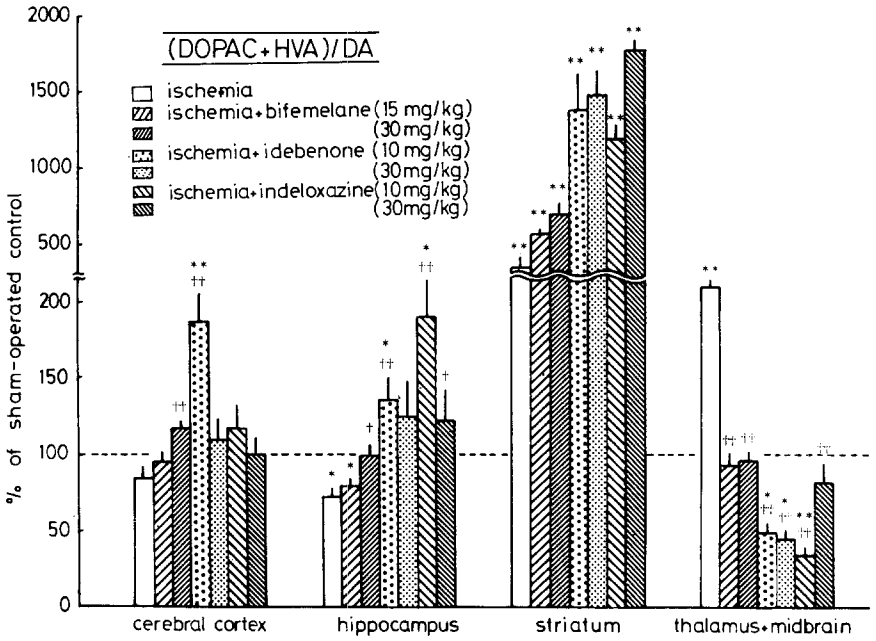

Fig. 1. Effects of bifemelane, idebenone and indeloxazine on ischemia-induced changes in DA turnover [(DOPAC + HVA)/DA] in four brain regions. Data are means ± SEM (N = 6). * $p < 0.05$ and ** $p < 0.01$ vs. sham-operated controls. + $p < 0.05$ and + + $p < 0.01$ vs. ischemic controls

sham-operated group, in any brain region, between the saline-treated and drug-treated gerbils (Table 2). In the ischemic group, NA concentration was markedly lower in all brain regions in the saline-treated gerbils. Bifemelane dose-dependently blocked the ischemia-induced depletion of NA in the cerebral cortex, hippocampus and striatum. In the thalamus + midbrain, idebenone and indeloxazine results in higher NA concentrations.

Serotonin (5 HT) and 5-hydroxyindoleacetic acid (5 HIAA) concentrations

Animals with cerebral ischemia had lower 5 HT concentrations in both the cerebral cortex and hippocampus, higher concentrations in the striatum, and there were no differences in the thalamus + midbrain. The level of the 5 HT metabolite, 5 HIAA in the cerebral cortex and thalamus + midbrain were significantly higher in the ischemic gerbils (Table 3).

Bifemelane dose-dependently blocked the ischemia-induced changes in 5 HT concentration in both the cerebral cortex and the striatum. Idebenone and indeloxazine, in contrast, resulted in markedly higher 5 HT and 5 HIAA levels in the hippocampus, and higher 5 HT levels in the striatum and thalamus + midbrain. Idebenone-treated animals (30 mg/kg) had higher 5 HIAA levels in the thalamus + midbrain and in indeloxazine-treated animals there was no ischemia-induced increase in 5 HIAA level in the cerebral cortex (Table 3). The drugs did not change 5 HT or 5 HIAA levels in the sham-operated group (Table 3).

The turnover rates (5 HIAA/5HT) of 5 HT are shown in Fig. 2. The ischemic group had markedly less 5 HT turnover in the striatum, but more 5 HT turnover in three other brain regions. After bifemelane, the indices of on 5 HT turnover in the cerebral cortex, hippocampus and thalamus + midbrain were normal. Indeloxazine, on the other hand, was associated with markedly less 5 HT turnover in all brain regions, and idebenone also depressed 5 HT turnover in all brain regions except cerebral cortex (Fig. 2).

Discussion

The great sensitivity of the brain to decreases in oxygen has been well established. Cerebral ischemia results in marked and rapid changes in brain metabolism (Seisjo, 1981; Lust et al., 1985; Seisjo and Wieloch, 1985). Alterations in the levels and turnover of several neurotransmitters have been implicated in the pathogenesis of ischemic brain injury (Weinberger et al., 1985; Slivka et al., 1988), and drugs that directly act on neurotransmitter systems have been used to treat ischemia (Ogawa et al., 1989). Bifemelane, idebenone and indeloxazine are used clinically to reduce apathy and other emotional disturbances in patients with cerebrovascular disease (Rossignol and Margaret, 1980). However, the mechanisms of action and biochemical effects of these drugs are not well understood (Ogawa et al., 1988 b; Kakihana et al., 1984). Therefore, we compared the effects of bifemelane, idebenone and indeloxazine on the monoaminergic

Table 2. Effects of bifemelane, idebenone and indeloxazine on ischemia-induced changes in NA (ng/mg protein) in four brain regions

	Sham-operated group				Ischemic group						
	vehicle	bifemelane (30 mg/kg)	idebenone (30 mg/kg)	indeloxazine (30 mg/kg)	vehicle	bifemelane		idebenone		indeloxazine	
						(15 mg/kg)	(30 mg/kg)	(10 mg/kg)	(30 mg/kg)	(10 mg/kg)	(30 mg/kg)
Cerebral cortex	3.42 ± 0.24	3.72 ± 0.07	3.79 ± 0.09	3.56 ± 0.17	0.49 ± 0.10**	0.77 ± 0.23**	2.86 ± 0.41^{++}	0.60 ± 0.12**	0.71 ± 0.18**	0.70 ± 0.16**	1.26 ± 0.10**$^{+}$
Hippocampus	2.76 ± 0.29	2.66 ± 1.07	1.78 ± 0.36	1.93 ± 0.51	0.71 ± 0.08**	1.23 ± 0.21**	1.96 ± 0.44^{+}	0.50 ± 0.07**	0.69 ± 0.07**	0.24 ± 0.05**$^{++}$	0.68 ± 0.08**
Striatum	5.04 ± 1.34	8.94 ± 2.03	6.88 ± 0.10	5.75 ± 1.96	1.59 ± 0.37*	3.47 ± 0.75^{+}	4.23 ± 0.57^{+}	0.54 ± 0.01**	1.29 ± 0.40*	0.91 ± 0.19*	0.91 ± 0.16*
Thalamus + midbrain	1.78 ± 0.59	3.54 ± 1.07	3.54 ± 0.59	2.63 ± 0.64	0.64 ± 0.15*	0.87 ± 0.20	0.76 ± 0.17	3.08 ± 1.30^{+}	3.74 ± 0.49^{+}	1.23 ± 0.31	2.55 ± 0.34^{+}

Values are means ± SEM (n = 6)
* $p < 0.05$, ** $p < 0.01$ vs. sham-operated controls
$^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ vs. ischemic controls

Table 3. Effects of bifemelane, idebenone and indeloxazine on ischemia-induced changes in 5 HT and 5 HIAA (ng/mg protein) in four brain regions

	Sham-operated group				Ischemic group						
	vehicle	bifemelane (30 mg/kg)	idebenone (30 mg/kg)	indeloxazine (30 mg/kg)	vehicle	bifemelane		idebenone		indeloxazine	
						(15 mg/kg)	(30 mg/kg)	(10 mg/kg)	(30 mg/kg)	(10 mg/kg)	(30 mg/kg)
Cerebral cortex											
5 HT	2.36 ± 0.77	4.08 ± 0.47	4.15 ± 0.51	4.05 ± 0.56	0.89 ± 0.27*	3.16 ± 0.31**$^{++}$	5.65 ± 0.78^{++}	1.37 ± 0.27	2.22 ± 0.67	2.31 ± 0.44	4.76 ± 0.65
5 HIAA	0.72 ± 0.08	0.72 ± 0.07	0.58 ± 0.06	0.65 ± 0.08	1.26 ± 0.13**	1.28 ± 0.07**	1.84 ± 0.15**$^{+}$	1.23 ± 0.33	0.78 ± 0.06^{+}	0.56 ± 0.07^{++}	0.51 ± 0.05^{++}
Hippocampus											
5 HT	0.66 ± 0.03	0.63 ± 0.12	0.84 ± 0.23	0.86 ± 0.18	0.40 ± 0.04**	0.46 ± 0.05	0.80 ± 0.12	2-	4.92 ± 1.44	3.11 ± 0.57^{++}	6.15 ± 2.02**$^{+}$
5 HIAA	2.28 ± 0.17	1.86 ± 0.35	1.62 ± 0.76	1.78 ± 0.27	2.50 ± 0.32	2.67 ± 0.37	3.90 ± 0.50^{+}	.17 ± 0.64**$^{++}$	9-	5.37 ± 0.80^{+}	6.23 ± 0.90^{++}
								8.51 ± 0.33^{+}	.58 ± 1.65**$^{++}$		
Striatum											
5 HT	0.93 ± 0.26	1.16 ± 0.35	1.29 ± 0.30	1.57 ± 0.32	2.11 ± 0.23**	2.46 ± 0.43**	1.36 ± 0.13*	2.65 ± 0.60*	4.25 ± 0.17	5.47 ± 0.92^{++}	5-
5 HIAA	2.60 ± 0.76	0.83 ± 0.10	0.67 ± 0.07	0.78 ± 0.04	1.38 ± 0.26	1.88 ± 0.38	1.13 ± 0.15	0.58 ± 0.06	1.66 ± 0.53	2.03 ± 0.96	.51 ± 0.83**$^{++}$
											2.84 ± 0.26
Thalamus + midbrain											
5 HT	1.32 ± 0.20	1.38 ± 0.13	1.02 ± 0.18	0.82 ± 0.06	1.49 ± 0.23	1.82 ± 0.21	1.52 ± 0.23	19.3 ± 1.62^{++}	21.0 ± 2.49^{++}	13.6 ± 2.15^{+}	21.1 ± 3.40^{+}
5 HIAA	3.41 ± 0.43	3.15 ± 0.15	3.27 ± 0.12	3.41 ± 0.18	6.04 ± 0.98*	5.92 ± 0.47**	4.59 ± 0.79	5.77 ± 2.10	23.7 ± 6.86	1-	10.6 ± 1.18
										.41 ± 0.20**$^{++}$	

Values are means ± SEM (n = 6)
* $p < 0.05$, ** $p < 0.01$ vs. sham operated controls
$^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$ vs. ischemic controls

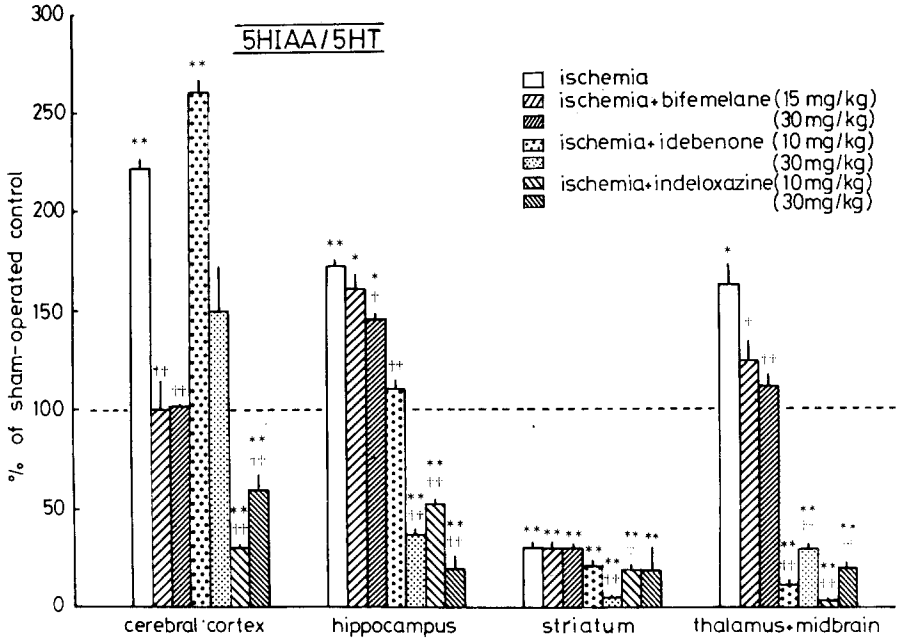

Fig. 2. Effects of bifemelane, idebenone and indeloxazine on ischemia-induced changes in 5 HT turnover (5 HIAA/5 HT) in four brain regions. Data are mean ± SEM (N = 6). * $p < 0.05$ and ** $p < 0.01$ vs. sham-operated controls. + $p < 0.05$ and + + $p < 0.01$ vs. ischemic controls

system in ischemic gerbils. These two doses used in the present study were observed the most pronounced effect of improvement on various ischemia-induced symptoms or changes in brain (Egawa et al., 1984; Kakihana et al., 1984; Yamamoto and Shimizu, 1987 a). In the sham-operated group, pretreatment with these drugs did not alter the concentration of any neurotransmitter in any region of the brain. This suggests that treatment with these agents has no effect on the brain under normal conditions.

It has been reported that pretreatment with idebenone attenuated the decrease in ACh level in the rat forebrain after a very short exposure to ischemia (Kakihana et al., 1984), but in our previous study idebenone did not inhibit the decrease in ACh concentration in ischemic gerbil brains (Ogawa et al., 1988 b). In the present study, idebenone hardly affected ischemia-induced changes in DA and 5 HT turnover to sham-operated control in all brain regions (Fig. 2). Indeloxazine also resulted in no effects on the changes in DA turnover in ischemic brain except in hippocampus and thalamus + midbrain (Fig. 1). The turnover of 5 HT in the ischemic gerbils was depressed in all brain regions after indeloxazine treatment (Fig. 2). These findings suggest that idebenone and indeloxazine have no essential action mechanism improving ischemia-induced symptoms in both DAergic and 5 HTergic systems.

It has been reported that bifemelane prolonged survival time and inhibited the reduction of NA and 5 HT contents in ischemic gerbil brains (Egawa et al.,

1984). Bifemelane alleviated the scopolamine-induced amnesia (Ogawa et al., 1988 a; Saito et al., 1985; Tobe et al., 1983). In addition, we have reported that bifemelane significantly and dose-dependently inhibited the ischemia-induced depression in the ACh concentration in the cerebral cortex, hippocampus, striatum and thalamus + midbrain (Ogawa et al., 1988 b). In the present study, bifemelane suppressed ischemia-induced changes in DA and 5 HT turnover in the cerebral ctex, hippocampus and thalamus + midbrain. These results suggest that bifemelane has an extensive effect on various neurotransmitter systems, especially in the cerebral cortex and hippocampus, and that it may be effective for treating cerebral ischemia (Egawa et al., 1984), hypoxia-anoxia (Tobe et al., 1983) and memory deficits (Ogawa et al., 1988 a) at least in part, because it interferes with the disruption of central cholinergic and monoaminergic systems. Furthermore, it has been reported that bifemelane and its metabolites have action of free radical scavenging *in vitro* (Liu et al., 1991). From the present results and those in previous reports, it is concluded that bifemelane may prevent brain damage due to recurrent ischemia, and that may be useful as a treatment for cerebrovascular diseases.

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