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PROTECTIVE EFFECT OF FLUNARIZINE AGAINST CEREBRAL HYPOXIA-ANOXIA IN MICE AND RATS

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The protective effect of flunarizine against cerebral hypoxia-anoxia was investigated with various experimental models in mice and rats. The effect of flunarizine was compared with those of cinnarizine, verapamil and pentobarbital. The oral treatment of animals with flunarizine resulted in a consistent and long-lasting protection against cerebral hypoxia-anoxia in all the models examined: Cytotoxic anoxia by KCN injection, hypercapnic anoxia induced by stopping artificial respiration, hypobaric hypoxia and normobaric hypoxia. The minimal effective dose of flunarizine was 1 to 20 mg/kg. The activity of flunarizine was 4 to 30 times as potent as that of cinnarizine and pentobarbital. Verapamil showed little or no protective effect. The mode of action of flunarizine was different from that of pentobarbital, which showed protection at anaesthetizing doses. These results indicate that flunarizine possesses a universal protective effect against cerebral hypoxia-anoxia, though the mechanism involved remains to be clarified. Hence, it is suggested that flunarizine might exert a beneficial effect on oxygen insufficiency of the brain resulting from cerebral ischemia.

Keywords— flunarizine; cinnarizine; verapamil; pentobarbital; anoxia; brain

INTRODUCTION

Flunarizine, 1-cinnamyl-4[bis-(*p*-fluorophenyl)methyl] piperazine dihydrochloride, has been known to possess peripheral and cerebral vasodilating properties *in vitro*[1,2] as well as *in vivo*.[3] It is also reported that flunarizine causes cerebrocortical vasodilation, followed by an increase in cerebrocortical oxygen tension.[4] In addition, flunarizine has been reported to have unique pharmacological actions such as improvement of red blood cell deformability[5] and depression of the vestibular system.[6] Clinically, flunarizine has been successfully used in the treatment of patients suffering from cerebral or peripheral vascular disorders.[7,8]

Recently, Waquier[9] demonstrated that flunarizine exhibited a potent protective effect against cerebral anoxia. The present study was conducted to confirm the results of Waquier[9] and furthermore to evaluate the effect of flunarizine against cerebral hypoxia-anoxia in several experimental models in comparison with cinnarizine, verapamil and pentobarbital.

MATERIALS AND METHODS

Experimental Design — Male dd mice and Wistar rats were used for the experiments. At a given period after the administration of the test compounds, the animals were subjected to various conditions of cerebral hypoxia-anoxia. The effect of the drug was estimated by comparing the value obtained after the drug administration with the control value. Statistical analysis was conducted by Fisher's exact probability test or Student's t-test.

Cytotoxic Anoxia by KCN Injection — Mice, weighing 22 to 28 g, were intravenously given 5.0 mg/kg of KCN saline solution. Inhibitory effects of the test compounds on the lethality were determined.

Hypercapnic Anoxia induced by Stopping Artificial Respiration — The method used was fundamentally similar to that previously described by Rosner *et al.*[10] Rats, weighing 220 to 280 g, were immobilized with gallamine triethiodide and were made to artificially respire *via* a trachea tube. Electrocorticograms (ECoG) were recorded with an electroencephalograph (Nihon Koden, ME135D). At a given period after the administration of the drug, the animals were subjected to hypercapnic anoxia by stopping artificial respiration. The cortical resistance time, which is defined as the time elapsed from the stopping of artificial respiration to the cessation of cortical activity, was determined.

Hypobaric Hypoxia — The method used was similar to that described by Nakanishi *et al.*[11] Mice, weighing 19 to 21 g, were placed inside a closed container and the inside pressure was rapidly reduced to 120 mmHg. The survival time, the time elapsing from the induction of hypobaric hypoxia to the respiratory failure of the animal, was determined.

Normobaric Hypoxia — Mice, weighing 18 to 20 g, were introduced into a glass container. They were exposed to a gas mixture of 4% oxygen and 96% nitrogen for 80 and thereafter they were brought back to the normal air. Inhibitory effects of the test compounds on the lethality of hypoxic challenge were determined.

Drugs — Flunarizine dihydrochloride (Janssen Pharmaceutica), cinnarizine (Kongo-Kagaku), verapamil (Isoptin®-hydrochloride, Knoll) and pentobarbital sodium (Nembutal®, Abbott).

RESULTS

Cytotoxic Anoxia by KCN Injection

A rapid injection of 5 mg/kg of KCN in mice produced convulsive seizures, followed by death within 5 min due to a respiratory block. The effects of the drugs tested are shown in Table I.

The treatment of animals with flunarizine resulted in a dose-related decrease in the mortality. The pretreatment for 2 h attained the maximal effect and the minimum effective dose of fluna-

TABLE I. *Protection by Several Drugs against Lethality caused by an Intravenous Injection of 5 mg/kg of KCN in Mice*

Treatment (route)	Pretreatment interval (h)	Dose (mg/kg)				
		10	20	40	80	160
Flunarizine (*p.o.*)	1	0/15[a)]	1/15	6/15*[b)]	8/15**	N.T.[c)]
	2	1/15	6/15*	11/15**	13/15**[b)]	N.T.
	4	2/15	4/15*	9/15**	13/15**	N.T.
Cinnarizine (*p.o.*)	1	N.T.	1/15	2/15	3/15*	11/15**
	2	N.T.	0/15	1/15	1/15	6/15*
	4	N.T.	0/15	2/15	1/15	8/15**
Verapamil (*p.o.*)	1	N.T.	0/10	0/10	0/10	3/10*
	2	N.T.	0/10	0/10	0/10	0/10
Pentobarbital (*p.o.*)	0,5	N.T.	0/10	2/10	8/10**	N.T.

a) Values are presented as No. survived/No. tested.
b) Statistically significant difference ($p < 0.05$, ** $p < 0.01$) from the control value (0/25).*
c) Not tested.

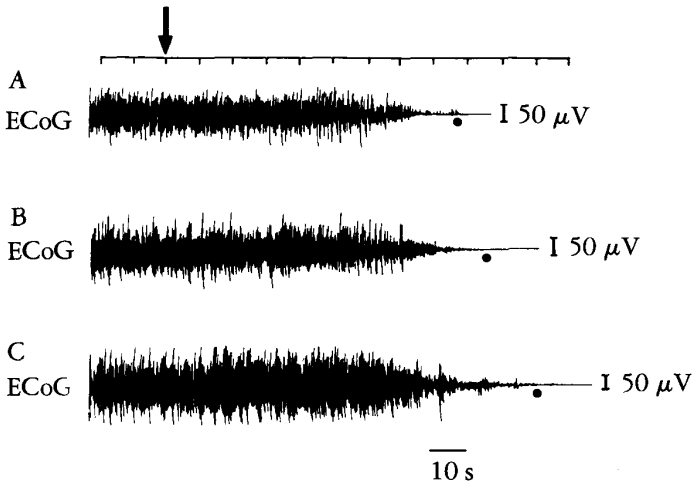

FIG. 1. *Protective Effect of Flunarizine on ECoG Response to Hypercapnic Anoxia in Rats*
A: control response; B: the response 3 h after 3 mg/kg of flunarizine (p.o.); C: the response 3 h after 10 mg/kg of flunarizine (p.o.) Artificial respiration was stopped at the arrow. Each closed circle (●) indicates the cessation of cortical activity.

rizine was 20 mg/kg (*p.o.*).

Cinnarizine and verapamil at their higher doses prevented the death and the maximal effect was observed 1 h after the administration.

Pentobarbital at 80 mg/kg (*i.p.*) which exhibits a strong anaesthetic activity prevented the death.

Hypercapnic Anoxia induced by Stopping Artificial Respiration

The typical ECoG activity of rats before and after stopping artificial respiration is presented in Fig. 1. Following the respiratory block, electrical silence on ECoG was seen and the average cortical resistance time for control animals was 76 s (Table II).

The treatment with 3 or 10 mg/kg of flunarizine significantly prolonged the cortical resistance time and the pretreatment for 3 h achieved the maximal effect.

Cinnarizine, whose maximal effect was attained 2 h after the administration, caused prolongation of the cortical resistance time at 30 mg/kg (*p.o.*). Verapamil showed little or no

TABLE II. *Effects of Several Drugs on Cortical Resistance Time of Rats subjected to Asphyxic Anoxia*

Treatment (route)	Dose (mg/kg)	Pretreatment interval (h)	Cortical resistance time (s)	(*n*)
Control			76.3±2.7 [a]	(8) [b]
Flunarizine (*p.o.*)	3	2	88.4±2.2*[c]	(6)
		3	95.5±5.9*	(6)
	10	2	92.6±3.3**[c]	(5)
		3	101.6±6.5**	(5)
Cinnarizine (*p.o.*)	10	2	82.4±5.2	(5)
	30	2	93.0±5.7*	(6)
Verapamil (*p.o.*)	10	2	80.0±3.0	(5)
	30	2	82.0±2.5	(5)
Pentobarbital (*i.p.*)	10	0.5	85.4±5.2	(5)
	30	0.5	100.5±5.7**	(6)

a) Values are presented as mean± SE.
b) Figures in parentheses indicate NO. of rats tested.
c) Statistically significant difference ($p < 0.05$, **$p < 0.01$) from the control.*

effect against hypercapnic anoxia. Pentobarbital at a dose of 30 mg/kg (*i.p.*) prolonged the resitance time.

Hypobaric Hypoxia

Exposure of mice to a hypobaric (120 mmHg) hypoxic condition caused convulsive seizures, followed by the death due to a respiratory failure. The survival time for control animals had an average of 110 s in the hypoxic condition tested (Table III).

Flunarizine at 1 to 10 mg/kg (*p.o.*) produced a significant prolongation of the survival time in hypobaric hypoxia.

While cinnarizine at a dose of 30 mg/kg (*p.o.*) was effective, verapamil showed no effect in the experiment. Pentobarbital at a dose of 30 mg/kg (*i.p.*) resulted in a significant marked prolongation of the survival time.

Normobaric Hypoxia

The mice which were exposed to a gas mixture of 4% oxygen adn 96% nitrogen died within several minutes and the mortality was 90% or more. The effects of the drugs on the lethality are shown in Table IV.

Flunarizine showed a marked protection of the animals from the death in normobaric hypoxia.

Cinnarizine at doses higher than 10 mg/kg (*p.o.*) was effective and 3 to 30 mg/kg (*p.o.*) of verapamil showed no effect in the experiment. Pentobarbital at 30 mg/kg significantly prevented the death.

DISCUSSION

It is well accepted that brain metabolism is highly dependent upon oxygen supply to the brain and that oxygen deprivation is one of the common and most damaging conditions affecting the brain function.[12] When oxygen supply to the brain becomes deficient, the cere-

TABLE III. *Effects of Several Drugs on Survival Time of Mice subjected to Hypobaric Hypoxia*

Treatment (route)	Dose (mg/kg)	Pretreatment interval (h)	Survival time (s)
Control			109.6±3.3 *a)*
Flunarizine (*p.o.*)	1	1	128.4±5.6* *b)*
		2	132.3±5.6** *b)*
	3	1	134.4±6.6**
		2	130.8±3.9**
	10	1	123.8±4.5*
		2	121.9±2.0**
Cinnarizine (*p.o.*)	10	1	119.7±4.6
	30	1	129.2±3.9**
Verapamil (*p.o.*)	3	1	103.0±3.9
	10	1	114.2±2.8
	30	1	104.9±3.2
Pentobarbital (*i.p.*)	10	0.5	112.6±5.5
	30	0.5	340.3±33.9**

a) Values are presented as mean ± SE for 10 animals.
b) Statistically significant difference ($p < 0.05$, **$p < 0.01$) from the control.*

bral function rapidly ceases or even in the survivors, neurological deficits occur, resulting from neuronal necrosis. Such oxygen deprivation can develop in many clinical situations such as cardiac arrest, traffic accidents, stroke or cerebral ischemia, *etc*. Against conditions of oxygen deprivation, barbiturates have widely been reported to have protective effect in a variety of animal experiments.[13-15] Moreover, barbiturates have recently been clinically used with success, though the usage is limited.[16,17] The protective effect of pentobarbital against cerebral anoxia was confirmed also by our present results.

In the present paper, the protective effect of flunarizine against cerebral hypoxia-anoxia, which was first reported by Waquier,[9] was compared with those of cinnarizine, verapamil and pentobarbital, using several animal models. Flunarizine showed a marked consistent protective effect in all of the models examined. The effective dose was different among individual experiments and this might be attributed to the difference in the degree of hypoxia-anoxia or to the species difference. However, through all our experiments, flunarizine was estimated to be about 4 to 30 times as potent as cinnarizine and pentobarbital. Verapamil showed little or no effect on the hypoxia-anoxia tested. These results and the previous work by Waquier suggest that flunarizine possesses a universal protective effect against cerebral hypoxia-anoxia.

The mode of action of flunarizine was different from that of pentobarbital, which showed protective effect only at its anaesthetizing doses. At doses at which flunarizine exhibited protection, it induced neither sedation nor motor depression. Therefore, the mechanism of protection by flunarizine cannot be ascribed to anaesthetizing action. The protective effect of flunarizine against hypoxia-anoxia might be, at least in part, attributed to the cerebral vasodilating action, accompanied by the increase in cerebrocortical pO_2.[4] However, papaverine, which is known to induce cerebral vasodilation as well as increase in cerebrocortical pO_2,[4] has been reported to exhibit no protective action against anoxia.[8] Moreover, flunarizine and verapamil differed in the action against cerebral hypoxia-anoxia, though both drugs possess novel Ca-antagonistic activity.[1,2,18] Accordingly, the protection by flunarizine seems not to be entirely explained by the cerebral vasodilating action

TABLE IV. *Protection by Several Drugs against Lethality caused by an Exposure to Normobaric Hypoxia in Mice*

Treatment (route)	Pretreatment interval (h)	Dose (mg/kg)				
		0.3	1	3	10	30
Flunarizine (*p.o.*)	1	1/12[a]	2/12	3/12	5/12**[b]	N.T.[c]
	2	2/12	7/12**	8/12**	8/12**	N.T.
Cinnarizine (*p.o.*)	1	N.T.	N.T.	2/12	6/12**	10/12**
	2	N.T.	N.T.	0/12	1/12	7/12**
Verapamil (*p.o.*)	1	N.T.	N.T.	3/12	1/12	0/12
Pentobarbital (*i.p.*)	0,5	N.T.	N.T.	N.T.	3/12	10/12**

a) Values are presented as No. survived/ No. tested.
*b) Statistically significant difference (** $p < 0.01$) from the control value (8/ 114).*
c) Not tested.

presumably due to Ca-antagonism. In this respect, some other unknown mechanism(s) could be involved.

Corkill *et al.*[19] reported that, in dogs whose middle cerebral artery was occluded, protection by pentobarbital occurred when the drug was given before or only 1 h after arterial occlusion. And current evidence suggests that the prevention in humans by means of anesthetizing dose of barbiturate is impractical unless the patient is anesthetized when ischemia occurs or unless the drug is given immediately after ischemia occurs.[20] As for flunarizine, it exerted, in rats and mice, a long-lasting and orally active prevention against cerebral hypoxia-anoxia with little affections on the central nervous system. Furthermore, recent investigations by Wauquier *et al.*[21] demonstrated that oral treatment with flunarizine reduced the extension of the infarct in rats after the unilateral occlusion of common carotid artery and exposure to hypoxia. We could, therefore, expect that the pretreatment with flunarizine might prevent the progress of cerebral stroke, especially in the recurrence of cerebral ischemia. This must, however, be confirmed by further experimental and clinical studies.

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