Protective Effect of *R*(−)-1-(Benzo[*b*]thiophen-5-yl)-2-[2-(*N*,*N*-diethylamino)ethoxy]ethanol Hydrochloride (T-588), a Novel Cerebral Activator, against Experimental Cerebral Anoxia

Satoshi Ono, Kazunori Kitamura, Mutsuko Maekawa, Kazunari Hirata, Motoko Ano, Wataru Ukai, Tetsuo Yamafuji and Hirokazu Narita

Research Laboratories, Toyama Chemical Co., Ltd., 2-4-1, Shimookui, Toyama 930, Japan

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ABSTRACT—Effects of *R*(−)-1-(benzo[*b*]thiophen-5-yl)-2-[2-(*N*,*N*-diethylamino)ethoxy]ethanol hydrochloride (T-588) on normobaric hypoxia, histotoxic anoxia by KCN and complete ischemia by decapitation were investigated in mice. T-588 (30-100 mg/kg, p.o.) showed a significant and dose-dependent prolongation of the survival time in all of the models studied. Bifemelane (100-300 mg/kg, p.o.) was also protective against all the models. Tacrine was protective against hypoxia but had no effect on anoxia and ischemia. Imipramine was protective against anoxia, but shortened the survival time of hypoxic mice. It had no effect on ischemia. The anti-hypoxic effect of T-588 was completely inhibited by pretreatment with scopolamine (1 mg/kg, i.p.), while the anti-anoxic effect was partially inhibited. Its effect on the ischemia was not affected by scopolamine. Hypoxia decreased the cerebral contents of ATP, phosphocreatine and glucose and increased the contents of lactate in mice. T-588 had no effect on these changes. Bifemelane prolonged pentobarbital-induced sleeping time in mice with the doses inducing anti-anoxic action, but T-588 did not. These results suggest that the activation of the CNS cholinergic system is involved as one of the mechanisms for the anti-anoxic action of T-588.

Keywords: T-588, Tacrine, Cerebral activator, Anti-anoxic action, Cholinergic function

Senile dementia of the Alzheimer type and multi-infarct dementia are considered to be major problems of contemporary societies in every part of the world. These days a number of compounds have been proposed for the treatment of senile cognitive disorder. Our extensive research on new types of cognitive enhancers led us to find a series of pharmacologically active 1,4-ethandiol derivatives. Among them, *R*(−)-1-(benzo[*b*]thiophen-5-yl)-2-[2-*N*,*N*-diethylamino)ethoxy]ethanol hydrochloride (T-588, Fig. 1), which produced excellent protection against normobaric hypoxia and ameliorated electroconvulsive shock-induced amnesia in mice as reported in a preliminary report (1), was finally selected for further investigations.

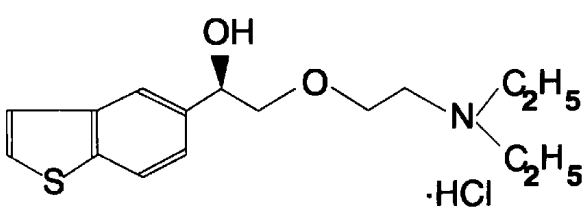

Fig. 1. Chemical structure of T-588.

It is well known that the function of the brain depends on the oxidation of glucose and that oxygen deprivation induced by hypoxia, anoxia or ischemia depresses brain function. Memory and learning are also impaired by hypoxia in animals (2, 3) and humans (4). Cerebral hypoxia and ischemia in animals have been widely used for the evaluation of cerebral improving drugs.

In the present study, we examined the cerebral protective effect of T-588 in comparison with other cerebral activators and estimated its pharmacological properties.

MATERIALS AND METHODS

Animals

The animals used were male ddY strain mice (19-30 g) purchased from Japan SLC, Inc. (Hamamatsu). They

were housed under conditions of constant temperature and controlled illumination and given food and water ad libitum.

Compounds

T-588 and bifemelane hydrochloride were synthesized in our laboratory. T-588, bifemelane, tacrine (Aldrich, Milwaukee, WI, USA) and imipramine hydrochloride (Tofuranil®, Ciba-Geigy Japan, Takarazuka) were dissolved in distilled water. Sodium pentobarbital (Nenbutal®, Abbott Laboratories, North Chicago, IL, USA) and scopolamine hydrobromide (Wako Pure Chemical, Osaka) were dissolved in 0.9% saline. All compounds were given to the mice in a volume of 0.1 ml/10 g. The control mice were given the vehicle orally.

Normobaric hypoxia in mice

Mice were put into a 300-ml glass container, and then a mixture of 4% oxygen and 96% nitrogen gas was introduced into the container. The gas mixture was continuously passed through the container at a flow rate of 5 l/min. The time when the respiratory arrest occurred was recorded as the survival time. Compounds were orally administered 30 min before the hypoxia.

Histotoxic anoxia by KCN in mice

Histotoxic anoxia was produced by an intravenous injection of 4 mg/kg KCN (0.1 ml/10 g). The time between KCN injection and the cessation of respiration was recorded. Compounds were orally administered 30 min before the KCN injection.

Complete ishemia by decapitation in mice

Cerebral ischemia was produced by decapitation. Compounds were orally administered 30 min before the decapitation. The time between decapitation and cessation of respiration was recorded as the gasping duration.

Determination of glycolytic metabolites and high-energy phosphate

Mice pretreated with test compounds and/or hypoxia were sacrificed by freezing the whole body with liquid nitrogen. The whole brain (without cerebellum) was taken out on a dry ice block. The brains were weighed, and homogenized in 10 vol. of 1 N $HClO_4$ with a glass homogenizer. The homogenate was centrifuged at $2000 \times g$ for 20 min, and the supernatant was used for assays of glycolytic metabolites and high-energy phosphates. ATP, glucose and phosphocreatine were determined by the method of Bergmeyer et al. (5). Lactate was determined by the method of Gutmann and Wahlefeld (6).

Pentobarbital-induced sleep in mice

Compounds were orally administered 30 min before the intraperitoneal injection of 55 mg/kg pentobarbital. The duration of sleep was measured as the time from the onset of loss of the righting reflex by pentobarbital treatment until the righting reflex was regained.

Statistical analysis

The results are expressed as the mean ± S.E.M. Statistical significance was assessed by one-way analysis of variance followed by Dunnett's test. In the case of the pretreatment with scopolamine, Student's *t*-test was used.

RESULTS

Effect on normobaric hypoxia in mice

The effects of T-588 and reference compounds against hypoxia are shown in Table 1. T-588 (30 – 100 mg/kg) significantly prolonged the survival time of mice subjected to hypoxia. A similar protective action against hypoxia was observed in mice treated with tacrine (30 mg/kg) and bifemelane (100 and 300 mg/kg). Imipramine (60 mg/kg) significantly shortened the survival time.

Table 1. Effects of T-588 and reference compounds on the normobaric hypoxia in mice

Compound	Dose (mg/kg, p.o.)	Survival time (sec)
Control	—	108 ± 9
T-588	3	124 ± 15
	10	154 ± 19
	30	203 ± 23**
Control	—	82 ± 3
T-588	30	158 ± 21**
	60	204 ± 17**
	100	208 ± 14**
Control	—	89 ± 3
Tacrine	3	91 ± 3
	10	120 ± 14
	30	164 ± 27**
Control	—	83 ± 3
Imipramine	10	90 ± 6
	30	79 ± 3
	60	65 ± 3**
Control	—	87 ± 3
Bifemelane	30	91 ± 6
	100	108 ± 10
	300	132 ± 19*

Compounds were administered 30 min before the hypoxia. Values represent the mean ± S.E.M. (n = 10). *P < 0.05, **P < 0.01, compared with each control value.

Table 2. Effects of T-588 and reference compounds on the KCN-induced anoxia in mice

Compound	Dose (mg/kg, p.o.)	Survival time (sec)
Control	—	35.3±1.7
T-588	3	39.4±1.4
	10	41.3±2.1
	30	51.0±2.8**
Control	—	30.1±1.0
T-588	30	51.4±6.8*
	60	62.4±5.0**
	100	70.9±6.8**
Control	—	33.9±1.4
Tacrine	3	32.6±0.6
	10	31.7±1.6
	30	37.6±2.0
Control	—	31.3±1.4
Imipramine	10	37.0±2.4
	30	35.9±1.6
	60	47.6±2.7**
Control	—	32.9±1.6
Bifemelane	30	33.7±1.1
	100	41.4±4.7
	300	53.7±5.3**

Compounds were administered 30 min before the KCN injection. Values represent the mean±S.E.M. (n=7). *P<0.05, **P<0.01, compared with each control value.

Table 3. Effects of T-588 and reference compounds on decapitation-induced gasping in mice

Compound	Dose (mg/kg, p.o.)	Gasping duration (sec)
Control	—	20.1±0.9
T-588	3	20.9±0.5
	10	22.5±0.9
	30	27.5±1.2**
Control	—	17.6±0.9
T-588	30	23.1±1.0**
	60	34.5±1.6**
	100	36.3±0.8**
Control	—	18.4±1.7
Tacrine	3	18.5±0.9
	10	19.0±1.2
	30	19.5±1.2
Control	—	20.0±0.5
Imipramine	10	19.6±0.7
	30	21.4±0.5
	60	23.1±1.2*
Control	—	19.9±0.5
Bifemelane	30	19.6±0.8
	100	24.1±1.2*
	300	26.6±1.3**

Compounds were administered 30 min before induction of decapitation. Values represent the mean±S.E.M. (n=8). *P<0.05, **P<0.01, compared with each control value.

Effect on KCN-induced anoxia in mice

T-588 exerted a significant prolongation of the survival time at 30–100 mg/kg. Bifemelane (300 mg/kg) and imipramine (60 mg/kg) also prolonged the survival time. Tacrine (3–30 mg/kg) showed no effect on the survival time (Table 2).

Effect on decapitation-induced gasping in mice

T-588 produced a significant prolongation of the duration of gasping at 30–100 mg/kg. Bifemelane (100 and 300 mg/kg) and imipramine (60 mg/kg) also prolonged the gasping duration. Tacrine (3–30 mg/kg) had no effect on the gasping duration (Table 3).

Effect of scopolamine treatment on protective action of T-588 against cerebral anoxia

Scopolamine (1 mg/kg, i.p.) had no effect on the normobaric hypoxia, KCN-induced anoxia or the gasping caused by decapitation in mice. Scopolamine was injected 10 min before administration of T-588 (30 mg/kg, p.o.). The results are shown in Fig. 2. The anti-hypoxic effect of T-588 was completely inhibited by scopolamine, while the

Table 4. Effect of T-588 on the changes in cerebral energy metabolites in hypoxic mice

Treatments		μmol/g wet wt.			
Compound	Hypoxia	ATP	PCr	Glucose	Lactate
Normal (vehicle)	−	2.82±0.03*	3.22±0.12**	1.04±0.02**	2.07±0.11**
Control (vehicle)	+	1.84±0.31	1.22±0.21	0.36±0.06	8.98±0.23
T-588 (30 mg/kg, p.o.)	+	2.16±0.31	1.38±0.21	0.34±0.02	8.78±0.17

Compound was administered 30 min before the hypoxia. Mice were sacrified 90 sec after the hpoxia. Values represent the mean±S.E.M. (n=6). *P<0.05, **P<0.01, compared with control value. PCr: Phosphocreatine.

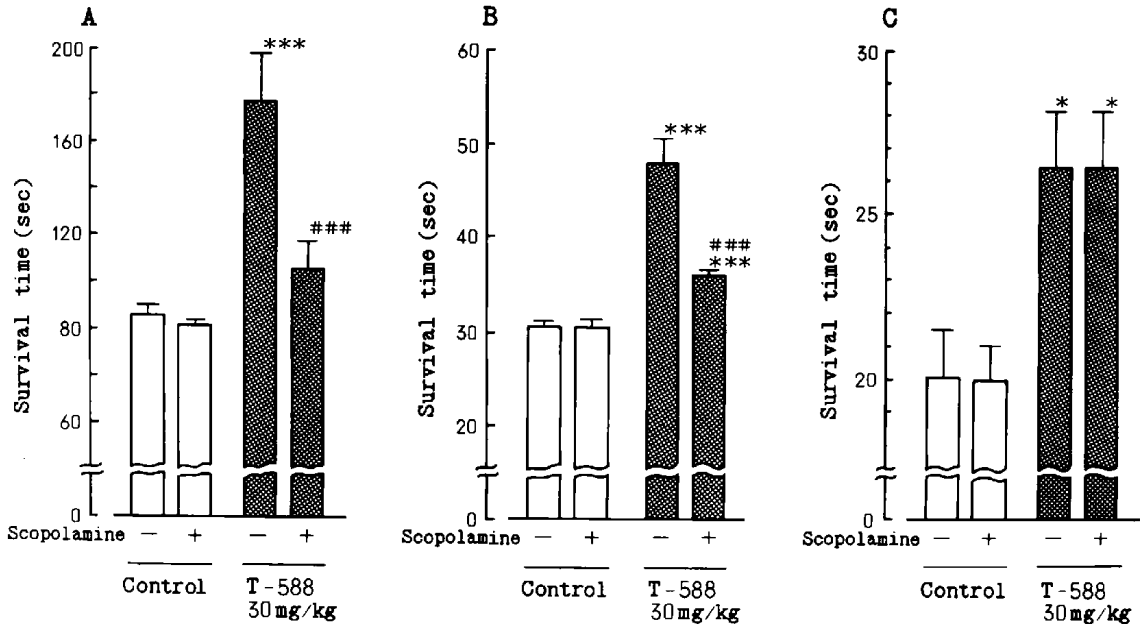

Fig. 2. Antagonistic effect of scopolamine on the effect of T-588. A: Hypoxia (n=10), B: KCN-induce anoxia (n=7), C: Complete ischemia (n=8). Each column with a vertical bar represents the mean±S.E.M. *P<0.05, ***P<0.001, compared with control value. ###P<0.001, compared with the value of T-588 alone. T-588 and scopolamine were administered at 30 and 40 min before the anoxic treatment, respectively.

anti-anoxic effect was partially inhibited. On the other hand, the effect of T-588 on decapitation-induced gasping was not affected by scopolamine.

Brain energy levels in hypoxic mice

As shown in Table 4, mean ATP, phosphocreatine and glucose levels in the control mice were respectively decreased by hypoxia treatment to 65%, 38% and 35% compared to those in the normal mice, and the mean lactate level was increased to 331%. These hypoxia-induced metabolic changes were not affected by T-588 (30 mg/kg).

Table 5. Effects of T-588 and reference compounds on pentobarbital-induced sleep in mice

Compound	Dose (mg/kg, p.o.)	n	Sleeping time (min)
Control	—	10	64.0± 5.6
T-588	10	10	64.7± 4.7
	30	10	69.6± 4.6
Control	—	10	77.0± 6.3
T-588	100	10	73.0± 4.8
Control	—	10	60.6± 6.1
Imipramine	30	10	89.1±11.2
	60	10	108.1± 9.8**
Control	—	10	76.5± 5.9
Bifemelane	30	10	122.7±10.4**
	100	10	161.8±11.0**

Compounds were administered 30 min before pentobarbital (55 mg/kg, i.p.). Values represent the mean±S.E.M. **P<0.01, compared with each control value.

Effect on pentobarbital-induced sleep in mice

T-588 had no effect on pentobarbital-induced sleep at 10–100 mg/kg. Bifemelane (30 and 100 mg/kg) and imipramine (60 mg/kg) significantly prolonged the sleeping time (Table 5).

DISCUSSION

It is reported that sedative drugs, such as barbiturate and diazepam (7–10) possess anti-anoxic effects. The mechanism of the anti-anoxic activity of these drugs have been explained by cerebral metabobolic depression or suppression of the energy demand (9, 11, 12). In this study, T-588 prolonged the survival time in all of the models studied, and it had no effect on pentobarbital sleeping. Therefore, the anti-anoxic effects of T-588 are not attributable to central depressant activities, and the mode of the anti-anoxic actions of T-588 is different from those of sedative drugs.

It is known that the brain is particularly vulnerable to reduction of its energy supply and that cerebral metabolic enhancers possess anti-hypoxic activities (13). Furthermore, Gibson and Duffy (14) reported that even mild

hypoxia, showing no alteration of the concentrations of ATP, impaired acetylcholine synthesis. Scremin and Scremin (15) reported that physostigmine, a cholinesterase inhibitor, prolonged the survival time of mice subjected to hypoxia. The present observation that tacrine, a cholinesterase inhibitor, prolonged survival time of hypoxic mice, was in good agreement with the report described by Scremin and Scremin (15). It is therefore possible for us to consider that the function of acetylcholine in the central nervous system might play a role as a neurotransmitter in the mechanism for survival under the hypoxic situation.

Thus, we examined whether T-588 may enhance the central cholinergic function. The present experimental results show that the protective effect of T-588 on hypoxia was completely inhibited by scopolamine (1 mg/kg). Furthermore, T-588 did not prevent hypoxia-induced changes in cerebral energy metabolism. These findings suggest that T-588 might improve the failure of cholinergic function rather than the insufficiency of energy supply in the brain under the hypoxic condition.

On the other hand, both imipramine and bifemelane are known to possess common inhibitory activities of reuptake of noradrenaline and serotonin (16); however, they showed different actions: bifemelane prolonged the survival time of hypoxic mice, while imipramine shortened it. Bifemelane has been reported to inhibit the decreases of brain acetylcholine levels in hypoxic rats (17), and ameliorate the reduction in the synthesis of acetylcholine in anemic hypoxia (18). It is known that imipramine possesses anti-cholinergic activity (19, 20). In our study, scopolamine did not affect the survival time of hypoxic mice at the dose of 1 mg/kg, i.p. (Fig. 2), but it significantly shortened the survival time at the dose of 3 mg/kg, i.p. (data not shown). Considering that scopolamine shortened and tacrine increased the survival time under hypoxia, anti-cholinergic action is assumed to explain the shortening effect of imipramine. Therefore, these differences in results can be considered to stem from the difference in the pharmacological profile of these drugs.

KCN inhibits the terminal oxidase enzyme cytochrome oxidase and subsequent breakdown of cellular metabolism, leading to a disruption of cellular metabolism (21, 22). In this study, T-588, bifemelane and imipramine prolonged the survival time of mice subjected to KCN-induced anoxia. Tacrine prolonged the survival time of hypoxic mice, while it had no effect on anoxia. This observation might suggest that the activation of the CNS cholinergic system is not sufficient to prolong the survival time of anoxic mice. Bifemelane and imipramine stimulate the central noradrenergic mechanism (16, 23, 24). Bifemelane inhibits the reduction of noradrenaline and serotonin content induced by bilateral carotid ligation (25). Therefore, it is considered that the activation of CNS monoaminergic function is involved as one of the mechanisms for the anti-anoxic action of bifemelane and imipramine. On the other hand, Gibson and Blass reported that the impairment of acetylcholine synthesis occurred in anoxic brain (26). Gibson et al. reported that KCN reduced potassium-stimulated synaptosomal acetylcholine release (27). The anti-anoxic action of T-588 was partially inhibited by scopolamine. This result suggests that the anti-anoxic action of T-588 might be related, at least partly, to enhancement of the cholinergic function. However, considering that tacrine shows no effect on anoxia, further investigation must be performed before making definitive conclusions regarding the mechanism(s) of the anti-anoxic action of T-588.

In cases of complete ischemia by decapitation, it is considered that the prolongation of the survival time, as induced by drugs may relate to the protection of nerve cells in the respiratory center (28). T-588 prolonged the gasping duration of mice subjected to complete ischemia. A similar protection against ischemia was also observed by the administration of bifemelane and imipramine. On the other hand, tacrine showed no effect. The protective action of T-588 was not affected by scopolamine. These results suggest that T-588 may preserve the integrity of the nerve cells of the respiratory center.

The protective action of bifemelane was similar to that of T-588, while the mode of the anti-anoxic actions of T-588 is different from that of bifemelane in that bifemelane prolonged the pentobarbital-induced sleeping time in mice with the doses inducing anti-anoxic actions, but T-588 did not. In future reports, further data indicating that T-588 exerted facilitatory effects on memory impairment in rats with cerebral infarction and anoxia-induced amnesia in mice will be described.

In conclusion, T-588 possessed protective effects against cerebral hypoxia, anoxia and ischemia without inducing CNS depression. The mode of the anti-hypoxic or anti-anoxic actions of T-588 might be considered to be related, at least partly, to enhancement of the cholinergic function.

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