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Effects of indeloxazine hydrochloride on cognitive disturbance in cycloheximide-treated mice

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Abstract—The effects of indeloxazine hydrochloride [(±)-2-[(inden-7-yloxy)methyl]morpholine hydrochloride] on cognitive disturbance in mice subjected to cycloheximide have been examined. Indeloxazine ameliorated cycloheximide-induced amnesia in mice, indicating a facilitatory effect of this drug on cerebral functions. Piracetam, Ca-hopantenate, dihydroergotoxine and viloxazine did not show significant effect on the amnesia. These results suggest that indeloxazine possesses a wider pharmacological profile than piracetam, Ca-hopantenate, dihydroergotoxine and viloxazine in anti-amnesic activities.

Impairment of neurotransmitter systems and decrease in cerebral metabolism and blood flow are well known in patients with cerebral vascular diseases, head injury, senile dementia, and other organic brain syndromes (Nilson & Ponten 1977; Jellinger et al 1978; Bes et al 1978). These patients frequently show multiple symptoms such as reduced spontaneity, emotional and cognitive disturbances, and others (Wood 1975; Ohtomo 1981). Therefore, drug enhancing cerebral functions would be useful in the treatment of various symptoms.

It is well known that scopolamine (an anticholinergic drug), cycloheximide (a protein inhibitor), anoxia, and cerebral ischaemia induce cognitive disturbance in animals (Rainbow 1978; Hunter et al 1977; Jensen & De Fine Olivarius 1980). Therefore, these amnesic models have been used for evaluating the effect of drugs on cerebral functions (Cumin et al 1982; Yamamoto & Shimizu 1987a, b). We have already reported that indeloxazine ameliorates cognitive disturbances induced by anoxia, cerebral ischaemia, and scopolamine in rodents. In addition, we found that the pharmacological profile of indeloxazine was wider than those of piracetam, dihydroergotoxine, and calcium hopantenate, drugs currently used in the therapy of cerebral vascular diseases (Table 1) (Yamamoto & Shimizu 1987 a,b; Yamamoto et al 1987a,b). The present study describes the effect of indeloxazine on cognitive disturbances in mice subjected to cycloheximide. Comparisons were conducted with the following drugs: dihydroergotoxine (cerebral vasodilator), piracetam and calcium hopantenate (cerebral metabolic enhancer) and viloxazine (antidepressant; the chemical structure of viloxazine, [2-(2-ethoxyphenoxymethyl) tetrahydro-1,4-oxazine hydrochloride], is similar to indeloxazine) (Greenwood 1975).

Table 1. Effects of indeloxazine, piracetam, calcium hopantenate, dihydroergotoxine and viloxazine on cognitive disturbances in rodents.

Amnesic model	Indelo.	Piracetam	Hopa.	Dihydro.	Vilo.	Ref.
[previous study]						
Scopolamine (passive avoidance)	+	(−)	−	−	−	a
Anoxia						
(passive avoidance)	+	(−)	+	−	−	b
(active avoidance)	+	(+)	+	−	−	b
Cerebral ischaemia						
(passive avoidance)	+	(−)	−	−		c,d
[present study]						
Cycloheximide (passive avoidance)	+	−	−	−	−	

Indelo.: Indeloxazine, Hopa.: Ca-hopantenate, Dihydro.: Dihydroergotoxine, Vilo.: Viloxazine, +: Effective, −: Not effective. a: Yamamoto & Shimizu (1987a) b: Yamamoto & Shimizu (1987b) c: Yamamoto et al. (1987c) d. Yamamoto et al. (1987d), ( ): Yamamoto et al. (in preparation).

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Materials and methods

Studies were carried out using male ICR mice (about 35g), housed in group cages under 12 h light-dark conditions and given free access to laboratory chow and water.

Passive avoidance learning. Training was carried out according to the one-trial step-through procedure described by Jarvik & Kopp (1967). The apparatus consists of two compartments, one illuminated (14 × 10 × 10 cm; light (60W) with a height of 25 cm to top of chamber) the other dark (16 × 10 × 10 cm). The compartments were separated by a guillotine door (5 × 5 cm). Before acquisition training, the mouse received a single pretraining trial in which it was placed in the illuminated compartment and, 10 s after, the guillotine door was raised. After the mouse entered into the dark compartment, it was allowed to remain there for 10 s. In the acquisition trial, the mouse was placed in the illuminated compartment one wall of which had a hole, through which the mouse could enter into the dark compartment that had a grid on the floor. As soon as the mouse entered the dark compartment, a scrambled foot-shock (0·25 mA, 50 Hz) was delivered to the floor grid for 2 s. The mouse could escape from the shock only by stepping back into the illuminated side, thereafter, the mouse was returned to its home cage. Cycloheximide in a dose of 150 mg kg^{-1} was given subcutaneously 30 min before the training session. The drugs were orally or intraperitoneally administered 30 min before the training. About 24 h after training, the retention test was conducted by replacing the mouse in the illuminated compartment and latency of the step-through response was measured. The observation period for the behaviour was maximally 300 s in this test.

Spontaneous movement. The spontaneous movement of each animal was measured using an Animex counter (Animex III, Shimazu Co.) for 60 min after the oral administration of the test drug.

Drugs. Indeloxazine hydrochloride, viloxazine hydrochloride, and piracetam were prepared in the laboratories and dissolved in distilled water before use. The following drugs were obtained commercially: dihydroergotoxine mesylate (Sandoz Co.), calcium hopantenate (Tanabe Co.), and cycloheximide (Sigma Co.). Calcium hopantenate and cycloheximide were dissolved in distilled water and 0·9% NaCl solution, respectively. All drugs were administered with a volume of 0·1 mL/10 g to the mouse.

Results and discussion

Cycloheximide, in a dose of 150 mg kg^{-1} (s.c.), significantly shortened the latency of the step-through response in mice.

Table 2. Effects of indeloxazine on step-through passive avoidance response in cycloheximide-treated mice.

Treatment	Dose (mg kg^{-1})	Latency of Step-Through (s)
Control	—	48±28
Indeloxazine	1 po	97±36
	3	111±36*
	10	183±39**
	30	77±29
Control	—	48±28
Piracetam	30 po	17±3
	100	70±28
	300	67±28
Control	—	56±10
Ca-hopantenate	100 po	76±20
	300	103±24
	1000	56±21
Control	—	62±28
Dihydroergotoxine	0·1 ip	40±9
	0·3	74±36
	1	42±16
Control	—	62±28
Viloxazine	3 po	48±21
	10	77±38
	30	25±13

Each value represents the mean ± s.e. from 10 mice. Latency of step-through in normal mice was 236±31 s (N = 10, mean ± s.e.). Cycloheximide in a dose of 150 mg kg^{-1} was administered subcutaneously 30 min before training. The test drugs were also administered orally or intraperitoneally 30 min before training. Significantly different from the value for control group: * $P < 0.05$, ** $P < 0.01$ (Mann-Whitney U-test).

When indeloxazine (3 and 10 mg kg^{-1} p.o.) was administered 30 min before training, this shortened latency was ameliorated in cycloheximide-treated mice (Table 2). The test drugs were administered before training in order to evaluate the drug action in the acquisition and consolidation phases of the cognitive tasks. Thus, the present results indicate that indeloxazine seems to facilitate both the acquisition and consolidation of cognitive behaviour. In addition, spontaneous movement was not changed by the administration of indeloxazine in doses which effected cognitive disturbance (3 and 10 mg kg^{-1} p.o.; data not shown). Therefore, the results suggest that the effects of indeloxazine on cognitive disturbances may be due to the facilitation of cognitive function.

It has been reported that cycloheximide inhibits not only protein synthesis, but also monoamine synthesis and release (Flexner & Goodman 1975; Freedman et al 1982). The resulting deficiences are thought to disturb cognitive function in cycloheximide-treated rodents (Quinton 1971). In the present study, indeloxazine ameliorated cycloheximide-induced amnesia. This suggests the involvement of central monoaminergic nervous systems in improved cognitive ability by indeloxazine. The previous observations that indeloxazine antagonized the reserpine-induced hypothermia in mice (Tachikawa et al 1979) and increased monoamine contents of rat brain (Yamaguchi et al 1985), support the facilitatory effect of indeloxazine on central monoaminergic systems. Furthermore, facilitatory effects of indeloxazine on spontaneous EEG (Yamamoto & Shimizu 1987a) and brain energy metabolism (Yamamoto & Shimizu 1987b) may be attributable to, in part, the amelioration of the cognitive disturbances. Piracetam (500 mg kg^{-1} p.o.), a nootropic drug has been reported to improve cycloheximide-induced disruption of the memory of a passive avoidance response (Cumin et al 1982). In the present study, however, when piracetam (30–300 mg kg^{-1} p.o.) was administered 3 min before training, the shortened latency of step-through induced by cycloheximide was not changed. The reason why piracetam showed no influence on the acquisition of cognitive behaviour in this model may be due to different experimental procedures such as the intensity of foot shock. Dihydroergotoxine, calcium hopantenate, and viloxazine also showed no significant influence on shortened latency of step-through in cycloheximide-treated mice. Thus, it may be postulated that indeloxazine possesses wider pharmacological properties than piracetam, dihydroergotoxine and calcium hopantenate, as this has an ameliorating effect on cycloheximide-induced amnesia. Furthermore, the pharmacological profile of indeloxazine is distinct from viloaxazine as it relates to enhanced learning activities.

We have already reported that indeloxazine possesses anti-amnesic actions in rodents subjected to anoxia, cerebral ischaemia, and scopolamine (Yamamoto & Shimizu 1987a, b; Yamamoto et al 1987b) and that the pharmacological profile of indeloxazine is wider than that of dihydroergotoxine or calcium hopantenate (Table 1). The present study strongly supports our prevous pharmacological results using amnesic models as described in Table 1.

In conclusion, indeloxazine ameliorated cycloheximide-induced amnesia in mice, indicating a facilitatory effect of this drug on cerebral functions. The present results and previous pharmacological findings (Yamamoto & Shimizu 1987a,b; Yamamoto et al 1987b) suggest that indeloxazine may be useful in the treatment of various symptoms in patients with cerebral vascular diseases, head injury, senile dementia, and other organic brain syndromes.

References

Bes, A., Jauzac, P., Guell, A., Braak, L., Geraud, G. (1978) Hemodynamic and metabolic factors in human cerebral ischemia. Eur. Neurol. 17: 17–26

Cumin, R., Bandle, E. F., Gamzu, E., Haefely, W. E. (1982) Effects of the novel compound aniracetam (Ro 13-5057) upon impaired learning and memory in rodents. Psychopharmacology 78: 104–111

Flexner, L. B., Goodman, R. H. (1975) Studies of memory: inhibitors of protein synthesis also inhibit catecholamine synthesis. Proc. Natl. Acad. Sci. 72: 4660–4663

Freedman, L. S., Judge, M. E., Quartermain, D. (1982) Effect of cycloheximide, a protein synthesis inhibitor, on mouse brain catecholamine biochemistry. Pharm. Biochem. Behav. 17: 187–191

Greenwood, D. T. (1975) Animal pharmacology of viloxazine (Vivalan). J. Int. Med. Res. 3: Suppl. 3, 18–28

Hunter, B., Zornetzer, S. F., Jarvik, M. E., McGaugh, J. L. (1977) Modulation learning and memory: Effects of drugs influencing neurotransmitters. In: Iverssen LL, Iversen SD, Snyder SH (eds) Handbook of Psychopharmacology, vol 8. Plenum, New York, pp 531–577

Jarvik, M. E., Kopp, A. (1967) An improved one-trial passive avoidance learning situation. Psychol. Rep. 21: 221–224

Jellinger, K., Riederer, P., Kothbassere, P. (1978) Brain monoamines in human brain infarct. Acta Neuropath. 41: 173–176

Jensen, T. S., De Fine Olivarius, B. (1980) Transient global amnesia as a manifestation of transient cerebral ischemia. Acta Neurol. Scand. 61: 115–124

Nilson, B., Ponten, U. (1977) Experimental head injury in the rat. Part 2. Regional brain energy metabolism in concussive trauma. J. Neurosurg, 47: 252–261

Ohtomo, E. (1981) Clinical evaluation of Hopa in the treatment of cerebrovascular disorder. Clin. Eval. 9: 673–710 (in Japanese)

Quinton, E. E. (1971) The cycloheximide-induced amnesia gradient of a passive avoidance task. Psychonomic Sci 25: 295–296

Rainbow, T. C. (1978) Role of RNA and protein synthesis in memory formation. Neurochem. Res. 4: 297–312.

Tachikawa, S., Harada, M., Maeno, H. (1979) Pharmacological and biochemical studies on a new compound, 2-(7-indenyloxymethyl) morpholine hydrochloride (YM-08054-1), and its derivatives with potential antidepressant properties. Arch. Int. Pharmacodyn. Ther. 238: 81–95

Wood, R. A. (1975) Psychological medicine, organic illness. Bri. Med. J. 29: 723–726
Yamaguchi, T., Harada, M., Nagano, H. (1985) Effects of indeloxazine hydrochloride (YM-08054) on monoamines and their metabolites in rat brain. Biogenic Amines 3: 21–32
Yamamoto, M., Shimizu, M. (1987a) Cerebral activating properties of indeloxazine hydrochloride. Neuropharmacol. 26: 761–770
Yamamoto, M., Shimizu, M. (1987b) Effect of indeloxazine hydrochloride (YM-08054) on anoxia. Arch. Int. Pharmacodyn. 286: 272–281
Yamamoto, M., Shimizu, M., Sakamoto, N., Ohtomo, H., Kogure, H. (1987a) Protective effects of indeloxazine hydrochloride on cerebral ischemia in animals. Ibid. 286: 272–281
Yamamoto, M., Shimizu, M., Kawabata, S. (1987b) Pharmacological actions of indeloxazine hydrochloride (YM-08054), a cerebral activator. Comparison with pharmacological actions of some cerebral metabolic enhancers, and effect of a combination with some cerebrovasodilators and cerebral metabolic enhancers—Prog. Med. 7 (12): 141–154 (in Japanese)

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Employment of magnet-susceptible microparticles for the targeting of drugs

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Abstract—It has been demonstrated in cats that magnet-susceptible microspheres and liposomes containing neuromuscular blocking agents (dipyronium, pyrocurinum and diadonium) caused a deeper inhibition of the neuromuscular transmission in the limb placed in the magnetic field than in the control limb located beyond the field. The microparticles containing a short-acting neuromuscular blocking agent diadonium appeared to have the highest selectivity of action. The present method allows a pronounced neuromuscular block in a target area to be achieved without noticeable effect on P_{CO_2} of the exhaled air.

The search for ways of increasing the selectivity of action of drugs, of reducing their side effects and toxicity is one of the urgent problems of modern pharmacology. To this end, various techniques are used which enable targeted transport of agents to the target organs. This provides an increase in local concentration of agents in a particular area and at the same time a decrease of the total dose of the drug. Targeted transport of drugs within the vascular compartment (Gregoriadis 1984) can be achieved by pH-sensitive liposomes (Yatvin et al 1980a; Connor et al 1984) which can selectively release the agents during pH changes, e.g. in the inflammation focus; thermosensitive liposomes releasing their content in the presence of local hyperthermia (Yatvin et al 1980b; Sullivan & Huang 1985); and also by various techniques of drug microcapsulation with ferromagnetics (Widder et al 1978; Ibrahime et al 1983; Ovadia et al 1983; Markevicha et al 1980). Magnet-controlled transport has made it possible to achieve a selective increase in the concentration of antineoplastic agents (Tankovich et al 1985), X-ray contrast substances (Bykov et al 1987) and anti-bacterial agents (Prilutskaya 1985) in the target organ.

The present study was devoted to the possibilities of selective action of neuromuscular blocking agents enclosed in magnet-susceptible microparticles on the neuromuscular transmission in certain groups of muscles. For this purpose microcarriers were needed that would provide a high concentration of the agent in the target organ. Magnet-susceptible microspheres (MMS) on the basis of insoluble polyelectrolyte complexes and liposomes (ML) were employed.

Materials and Methods

The possibility of achieving a selective muscle relaxation with the help of neuromuscular blocking agents enclosed in HMS and ML was tested in cats, 2·7–3·5 kg. Tracheostomy was performed under ether anaesthesia and a catheter was introduced into the jugular vein. The animals were then given pentobarbitone sodium (30 mg kg^{-1}, i.v.). To prevent possible hypoxia associated with the action of neuromuscular blocking agents artificial ventilation was started in the animals before the experiment. The neuromuscular transmission in the gastrocnemius muscles of the cat was estimated according to the value of evoked potentials (EP) of muscles during electric stimulation of sciatic nerves by supramaximal rectangular pulses (0·5 Hz). EP were registered by bipolar needles, and 10 consecutive responses were averaged. One hind limb was placed in a magnetic field with strength of about 0·158 MA m^{-1}. The other hind limb, which remained beyond the field, served as control. EP in both limbs were registered continuously throughout the experiment.

Neuromuscular blocking agents with different duration of action were used: dipyronium (long-acting), pyrocurinum (medium-acting) and diadonium (short-acting) (Kharkevich 1986).

MMS from an insoluble polyelectrolyte complex were prepared by mixing in saline of the solutions of nucleic acid, polyethylenimine, neuromuscular blocking agent and ferroparticles. MMS were 0·7–2 μm in diameter and were injected i.v. One mL of suspension contained 350 μg of diadonium, 130 μg of pyrocurinum or 20 μg of dipyronium (single neuromuscular blocking dose per 1 kg b.w.). The animals were injected with 0·4 mL kg^{-1}.

The liposomes consisted of phosphatidylcholine and cholesterol in a molar ratio 5:2 and 7:2. The lipid film for ML was prepared in a rotor evaporator under vacuum. A buffer solution containing gelatine-stabilized ferroparticles, and neuromuscular blocking agents diadonium and dipyronium were then added to the lipid film. The resulting suspension was then sonicated under nitrogen at ice-melting temperature for 60-100 s. The size of ML ranged from 0·1 to 2 μm. The drugs not enclosed in liposomes were removed by dialysis for 24 h. The content of diadonium and dipyronium in 1 mL of the suspension was 100 μg mL^{-1} and 10 μg mL^{-1}, respectively.

The data were expressed as mean ± s.e.m. Statistical differences in the data were analysed by Student's *t*-test.

Results

It has been shown in control experiments that the magnetic field of 0·199 MA m^{-1} strength was without noticeable effect on the neuromuscular transmission at the chosen parameters of stimulation. Under these conditions the administration of neuromuscular blocking agents dipyronium, pyrocurinum and diadonium was associated with a decrease of the amplitude of potentials identical in both limbs of the animal. The injection of

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