*Pharmacology Biochemistry and Behavior*, Vol. 45, pp. 335–341, 1993
Printed in the U.S.A. 

0091-3057/93 $6.00 + .00


# Cerebral Activating Properties of Indeloxazine HCl and its Optical Isomers

MASAO SHIMIZU-SASAMATA,*[1]
MINORU YAMAMOTO* AND MASATOMI HARADA†

*Departments of *Pharmacology and †Biochemistry,*
*Medicinal Research Laboratories, Central Research Laboratories,*
*Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan*

Received 21 May 1992

SHIMIZU-SASAMATA, M., M. YAMAMOTO AND M. HARADA. *Cerebral activating properties of indeloxazine HCl and its optical isomers.* PHARMACOL BIOCHEM BEHAV 45(2) 335–341, 1993. – The cerebral activating actions of indeloxazine HCl and its optical isomers were evaluated in comparison with those of several selective monoamine uptake inhibitors. The effects of indeloxazine and its optical isomers on monoamine uptake were also determined. Indeloxazine was equipotent to the (−)-isomer in desynchronizing the spontaneous electroencephalogram (EEG) in both mature and aged rats and in accelerating recovery of consciousness induced by concussive head trauma in mice, whereas the (+)-isomer showed about 3 times less potent activity than indeloxazine and the (−)-isomer. The potency of indeloxazine and the (−)-isomer in inhibiting [$^{14}$C]norepinephrine ($^{14}$C-NE) uptake was approximately 25–30 times more potent than that of the (+)-isomer. Maprotiline and viloxazine, selective NE uptake inhibitors, also showed facilitatory actions in concussed mice. Selective 5-hydroxytryptamine (5-HT) uptake inhibitors such as citalopram, alaproclate, and zimeldine showed little effect. Indeloxazine, the (−)-, and the (+)-isomers facilitated passive avoidance learning behavior with a bell-shaped response curve in normal rats. The potency of the (+)-isomer in inhibiting [$^{14}$C]5-HT uptake was equipotent to those of both indeloxazine and the (−)-isomer. While citalopram, alaproclate, and zimeldine also facilitated the acquisition of learning behavior, maprotiline and viloxazine, as well as the dopamine uptake inhibitor amantadine, showed little influence on the latency of step-through behavior. Amitriptyline, with anticholinergic activity, impaired learning behavior. Indeloxazine, its optical isomers, citalopram, alaproclate, and zimeldine also ameliorated cycloheximide-induced disturbance of learning behavior in mice, while maprotiline and viloxazine showed little effect. These results indicate that indeloxazine and its optical isomers possess facilitatory effects on the CNS, and the pharmacological profile of indeloxazine and its optical isomers with NE and 5-HT uptake inhibitory activity were wider than those of other selective monoamine uptake inhibitors and the classical tricyclic antidepressant tested in the present study. In addition, it is suggested that central serotonergic and noradrenergic systems might be involved in learning behavior and central arousal action, respectively.

Indeloxazine HCl  Optical isomer  Selective 5-HT uptake inhibitors  Selective NE uptake inhibitors
Noradrenergic system  Serotonergic system  Learning behavior  Cerebral arousal action

INDELOXAZINE HCl (Fig. 1) (9) is a newly developed cerebral metabolic enhancer that showed not only facilitatory effects on the spontaneous electroencephalogram (EEG) and the acquisition of learning behavior but also protective effects against cerebral injuries resulting from anoxic states in a variety of pharmacological studies (19,23–25). In biochemical studies, indeloxazine was found to have inhibitory effects on the uptake of both [$^{14}$C]5-hydroxytryptamine ([$^{14}$C]5-HT) and [$^{14}$C]norepinephrine ([$^{14}$C]NE) into synaptosomes from the brain of the rat (7). Further, this drug attenuated the decreased levels of monoamines such as 5-HT and NE in affected brain regions of rats subjected to middle cerebral artery occlusion (19). Because of these effects in animals, indeloxazine has been tested for the treatment of various symptoms in patients with cerebral vascular diseases (15).

Indeloxazine is a racemic mixture with approximately equal proportions of the two enantiomers and its overall activity may be due to the sum of those of the individual enantiomers. Therefore, in the present study we aimed to determine whether the active isomer of indeloxazine is the (+)-isomer or the (−)-isomer. Indeloxazine possesses inhibitory effect on uptake of both NE and 5-HT and shows both central arousal activity and facilitatory effects on learning behavior. Therefore, we also examined the relationship between central monoaminergic neurotransmitters and brain functions such as central arousal activity and learning behavior using optical

[1] To whom requests for reprints should be addressed.

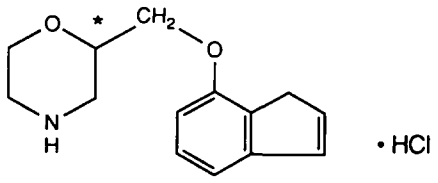

FIG. 1. Chemical structure of indeloxazine HCl. Asterisk denotes the point of asymmetry.

isomers of indeloxazine and selective monoamine uptake inhibitors.

## METHOD

### *Animals*

Male ICR mice weighing about 30 g (about 7 weeks old), male adult Wistar rats weighing about 300 g (about 17 weeks old), and male aged Wistar rats weighing about 350 g (about 21 months old) were purchased from Japan SLC, Inc. All animals were housed in cages under a 12 L : 12 D cycle and given laboratory chow and water ad lib. All experiments were performed between 9:00 a.m. and 6:00 p.m.

### *Spontaneous Eelectroencephalogram*

In a total of 20 adult rats and 20 aged rats, screw electrodes were implanted on the skull overlying the left cerebral cortex under pentobarbital anesthesia (50 mg/kg, IP). Seven days after implantation of the electrodes, pharmacological evaluations were conducted. The test drugs were administered IP. The spontaneous EEG was recorded for 90 min before drug administration to adapt rats to the new environment and, thereafter, for 120 min after drug administration without restraint. Spontaneous EEGs were analyzed every 10 min for delta-(2–3.75 Hz), theta-(4–7.75 Hz), and alpha-(8–12.75 Hz) wave components by means of a data analytic computer system (ATAC-450, Nihon Kohden Co.) via a polygraph (RM-6200, Nihon Kohden Co.). In preliminary studies, anesthetic agents such as pentobarbital decreased appearance rate of the theta wave component. On the other hand, methamphetamine, a psychostimulant, increased appearance rate of theta wave component, suggesting that theta wave component may be reflected in the conscious level in rats. Therefore, assessments of the effects of the test drugs were conducted by the incidence of the theta wave component (23).

### *Disturbance of Consciousness in Concussive Mice*

Experimental concussion was produced by a slight modification (23) of the methods described by Manaka and Sano (11). By holding the ears of the unanesthetized mouse, the head was placed on a piece of urethane foam rubber 1.5 cm thick. An acrylate weight containing lead (20.5 g, 19 mm in diameter and height) was dropped onto the head from about 18 cm height through a tube of 21 mm inner diameter. Immediately after this mechanical shock, mice usually manifested tonic convulsions and disturbance of consciousness and then remained motionless for some period. After a while, they showed recovery of the righting reflex and restless ambulation accompanied by exploratory behavior. The time from the physical insult to the onset of spontaneous movement was recorded as the disturbance time in consciousness (DT time). The test drugs were administered IV 10 min before the concussion. Immediately after the experiments, mice were killed by injection of pentobarbital.

### *Passive Avoidance Behavior*

The training of the passive avoidance behavior was conducted according to the one-trial step-through procedure described by Jarbik and Kopp (8). The apparatus consisted of two compartments, one illuminated [rat studies: 40 × 25 × 25 cm; mouse studies: 14 × 10 × 10 cm; both with light (60 W) at a height of 25 cm from the top of the chamber] and the other dark (rat: 20 × 15 × 25 cm; mouse: 16 × 10 × 10 cm). The compartments were separated by a guillotine door (rats: 10 × 7 cm; mice: 5 × 5 cm). Before the acquisition trial, the animal received a single pretraining trial in which it was placed in the illuminated compartment, 10 s after which the guillotine door was raised. After the rodent entered into the dark compartment, it was allowed to remain there for 10 s. In the acquisition trial, the animal was placed in the illuminated compartment, one wall of which had a hole through which the animal could enter into the dark compartment, which had a grid on the floor. As soon as the animal entered the dark compartment, a scrambled foot-shock (0.25 mA, 50 Hz) was delivered to the floor grid for 2 s. The animal could escape from the shock only by stepping back into the illuminated side. The animal was then returned to its home cage.

In the studies with rats, to evaluate the drug action in the acquisition and consolidation phases of the cognitive behavior test drugs were administered orally or IP 60 and 30 min before training, respectively. About 24 h after training, the retention test was conducted by replacing the rat in the illuminated compartment and measuring the latency of the step-through response. The observation period for the behavior was a maximum of 600 s in this test. In addition, we checked the latencies during the acquisition trial and proportion of rats per treatment returned to the safe side by the 2-s shock administration because it is important to exclude these factors to evaluate the drug action in the passive avoidance learning behavioral test.

In the studies with mice, cycloheximide in a dose of 150 mg/kg was given SC 30 min before the training session. Cycloheximide administration was used to impair learning and memory. The test drugs were orally administered 30 min before the training to evaluate the drug action in the acquisition and consolidation phases of the cognitive behavior. About 24 h after training, the retention test was conducted by replacing the rat in the illuminated compartment and measuring the latency of the step-through response. The observation period for the behavior was a maximum of 300 s in this test. In addition, we checked the latencies during the acquisition trial and proportion of mice per treatment returned to the safe side by the 2-s shock administration.

In both the studies with mice and rats, an increase in the step-through latency after drug treatment compared to the control group was defined as an "increase in learning and memory."

### *Uptake of Biogenic Amines*

Rats were decapitated and the whole brain or hippocampus immediately dissected. According to a slight modification (7) of the method described by Schacht and Heptner (18), the whole brain or hippocampus was homogenized in 0.32 M sucrose and put into differential centrifugation. After washing once with cold 0.32 M sucrose, the resulting pellet was used as crude synaptosomes. These synaptosomes were suspended at about 0.6 mg protein per 1 ml Krebs–Henseleit bicarbonate buffer containing 0.1 mM iproniazid, 11 mM glucose, 1.14 mM ascorbic acid, 0.067 mM EDTA, and 1.27 mM calcium chloride. For [$^{14}$C]5-HT uptake by the whole-brain synaptosomes, 2-ml aliquots of the synaptosomal suspension were

incubated with $10^{-7}$ M [$^{14}$C]5-HT for 2.5 min at 37°C in a shaking water bath after 3 min of preincubation in the presence or absence of test compounds. The mixture was quickly cooled in an ice bath and then filtered through a Whatman glass fiber disk GF/F (Whatman, Clifton, NJ). The disk was washed, dried, and transferred to a vial with 10 ml scintillation fluid for measuring radioactivity. [$^{14}$C]NE uptake by hippocampal synaptosomes (10) was also determined as described for [$^{14}$C]5-HT uptake except for the use of 1 ml of the reaction mixture.

The $IC_{50}$, the concentration of drug that inhibits the uptake reaction by 50%, was calculated from the dose-response curves by plotting percent inhibition on a probit scale as a function of log concentration.

### *Drugs*

Optical isomers of indeloxazine HCl (2-[(inden-7-yloxy)-methyl]morpholine HCl, YM-08054) were prepared by Dr. K. Niigata (Department of Chemistry, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan). Physicochemical properties of the optical isomers were as follows: *R*-(−)-indeloxazine: m.p. 142.5°C, $[\alpha]_D^{20}$ −4.94° ($c$ = 5, MeOH); S-(+)-indeloxazine m.p. 113.0°C, $[\alpha]_D^{20}$ +4.92° ($c$ = 5, MeOH). Viloxazine HCl and amitriptyline HCl were also prepared in our laboratories. Citalopram HBr (Cipramil®, H. Lundbeck & Co., Denmark), maprotiline HCl (Ludiomil®, Ciba-Geigy), and amantadine HCl (Nacalai Tesque) were obtained commercially from their respective sources. Alaproclate and zimeldine were the gift of Dr. B. Sjövall (Astra Research Centre). 5-[2-$^{14}$C]5-HT (56 mCi/mmol) and 1-[methylene-$^{14}$C]NE *d*-bitartrate (57 mCi/mmol) were purchased from the Radiochemical Center (Amersham, England). All drug solutions were made in either 0.9% w/v sodium chloride solution (saline), 0.5% methylcellulose (MC), or distilled water.

## RESULTS

### *Spontaneous EEG*

As shown in Fig. 2a, both indeloxazine and the (−)-isomer at a dose of 3 mg/kg IP significantly increased the incidence of the theta wave component while the (+)-isomer showed no influence on EEG arousal activity. The duration of the effect of indeloxazine and the (−)-isomer were approximately 60 and 90 min, respectively. On the other hand, indeloxazine, the (−)-isomer, and the (+)-isomer at a dose of 10 mg/kg IP significantly increased the incidence of the theta wave component (Fig. 2b). The effects of indeloxazine, the (−)-isomer, and the (+)-isomer lasted approximately 100, 120, and 20 min, respectively. Both indeloxazine and the (−)-isomer were about three times more potent and longer lasting than the (+)-isomer. These results suggest that all three drugs facilitated the spontaneous EEG in adult rats at same dosage. A similar activating effect was also observed in aged rats treated with administration of indeloxazine and its optical isomers at a dose of 10 mg/kg IP (Fig. 3).

### *Disturbance of Consciousness in Concussed Mice*

Indeloxazine and its (−)-isomer at IV doses of 3 mg/kg or higher significantly reduced the disturbance time in consciousness (DT time). The (+)-isomer of indeloxazine at 10 mg/kg significantly shortened the DT time. Based upon the minimum effective dose values, indeloxazine and its (−)-isomer were about three times more potent than the (+)-isomer (Table 1).

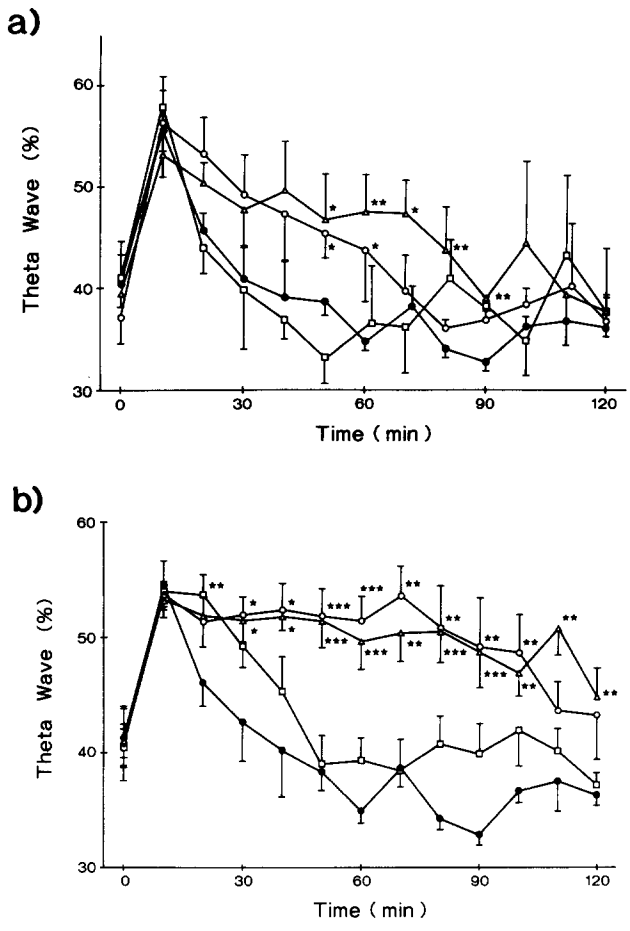


FIG. 2. Effects of indeloxazine HCl and its optical isomers on the theta wave component of spontaneous electroencephalogram (EEG) in adult rats. Each point represents mean ± SE from three to eight rats. Significantly different from the value for saline-treated group: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Student's $t$-test). (a) −●−, saline; −○−, indeloxazine (3 mg/kg, IP); −△−, (−)-indeloxazine (3 mg/kg, IP); −□−, (+)-indeloxazine (3 mg/kg, IP). (b) −●−, saline; −○−, indeloxazine (10 mg/kg, IP); −△−, (−)-indeloxazine (10 mg/kg, IP); −□−, (+)-indeloxazine (10 mg/kg, IP).

Maprotiline (3 mg/kg, IV) and viloxazine (10 mg/kg, IV), selective NE uptake inhibitors, also significantly shortened the DT time.

On the other hand, selective 5-HT uptake inhibitors such as citalopram, alaproclate, and zimeldine showed no influence on disturbance of consciousness.

### *Passive Avoidance Behavior in Rats*

As shown in Table 2, indeloxazine (2 mg/kg, IP, and 10 mg/kg, PO) significantly prolonged the latency of the step-through response in rats. The (+)- and (−)-isomers of indeloxazine at the same dose (2 mg/kg IP) also increased the step-through latency similar to the racemate. These effects were shown in a bell-shaped response curve. Citalopram, alaproclate, and zimeldine, selective 5-HT uptake inhibitors, also facilitated memory acquisition as defined by a significant increase in step-through latency. Maprotiline and viloxazine,

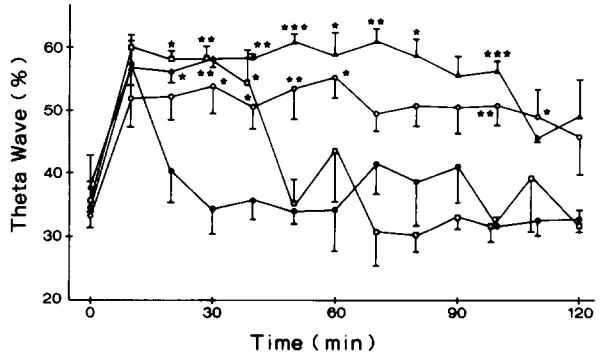


FIG. 3. Effects of indeloxazine HCl and its optical isomers on the theta wave component of spontaneous electroencephalogram (EEG) in aged rats. Each point represents mean ± SE from four rats. Significantly different from the value for saline-treated group: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Student's $t$-test). −●−, saline; −○−, indeloxazine (10 mg/kg, IP); −△−, (−)-indeloxazine (10 mg/kg, IP); −□−, (+)-indeloxazine (10 mg/kg, IP).

selective NE uptake inhibitors, and amantadine, a dopamine uptake inhibitor, had no effect on the step-through latency. In contrast, amitriptyline, a tricyclic antidepressant with anticholinergic activity, significantly decreased the step-through latency. In the acquisition trial, latency in drug-treated group was not significantly different from that in vehicle-treated group. The test drugs did not affect proportion of rats per treatment returned to the safe side by the 2-s shock administration during the acquisition trial (data not shown). Therefore, the effects of the test drugs on learning behavior are not due either to motor changes or changes in pain threshold.

*Passive Avoidance Learning in Cycloheximide-Treated Mice*

Cycloheximide at a dose of 150 mg/kg SC significantly shortened the latency of the step-through response in mice. Indeloxazine (10 mg/kg, PO), the (−)-isomer (10 mg/kg, PO), and the (+)-isomer (10 mg/kg, PO) significantly ameliorated cycloheximide-induced amnesia in mice (Table 3). These effects also gave a bell-shaped dose-response curve. The selective 5-HT uptake inhibitors such as citalopram, alaproclate, and zimeldine also prolonged the shortened latency in cycloheximide-treated mice at some dose. The selective NE uptake inhibitors, maprotiline and viloxazine, and the dopamine uptake inhibitor amantadine showed no effects in this amnesia model. In the acquisition trial, latency in drug-treated group was not significantly different from that in vehicle-treated group. The test drugs did not affect proportion of mice per treatment returned to the safe side by the 2-s shock administration during the acquisition trial (data not shown). Therefore, the effects of the test drugs on learning behavior are not due either to motor changes or changes in pain threshold.

*Uptake Inhibition of Biogenic Amines*

The uptake of [$^{14}$C]5-HT by synaptosomes from the whole brain was inhibited by indeloxazine and its individual optical isomers in a dose-dependent manner. As shown in Table 4, the $IC_{50}$ values of indeloxazine, the (−)-isomer, and the (+)-isomer were $7.1 \times 10^{-7}$, $6.5 \times 10^{-7}$, and $8.3 \times 10^{-7}$ M, respectively.

On the other hand, inhibitory effects of indeloxazine on uptake of [$^{14}$C]NE by synaptosomes from the hippocampus was found to be similar to those of the (−)-isomer, but the (+)-isomer was 25–30 times less potent. As shown in Table 4, the $IC_{50}$ values of indeloxazine, the (−)-isomer, and the (+)-isomer were $2.6 \times 10^{-7}$, $2.2 \times 10^{-7}$, and $68 \times 10^{-7}$ M, respectively.

Further, it has been reported that serotonin uptake in brain tissue is a stereoselective process because there are numerous examples of differences between effects of enantiomers and geometric isomers on 5-HT uptake (20). In the present study, however, there was no differences between the effects of the optical isomers of indeloxazine on 5-HT uptake, in contrast to other 5-HT uptake inhibitors such as alaploclate, zimelidine, and fluoxetine.

TABLE 1

EFFECTS OF INDELOXAZINE HCl, ITS OPTICAL ISOMERS, AND SELECTIVE MONOAMINE UPTAKE INHIBITORS ON THE DISTURBANCE OF CONSCIOUSNESS INDUCED BY CONCUSSIVE HEAD INJURY IN MICE

| Treatment | Dose (mg/kg, IV) | $n$ | Time (seconds)* |
|---|---|---|---|
| Saline | – | 11 | 482 ± 115 |
| Indeloxazine | 1 | 11 | 363 ± 92 |
| | 3 | 9 | 169 ± 35† |
| | 10 | 9 | 112 ± 28‡ |
| Saline | – | 9 | 550 ± 122 |
| (−)-Indeloxazine | 1 | 10 | 537 ± 122 |
| | 3 | 10 | 199 ± 37‡ |
| | 10 | 10 | 173 ± 40‡ |
| Saline | – | 10 | 474 ± 67 |
| (+)-Indeloxazine | 1 | 10 | 411 ± 105 |
| | 3 | 10 | 378 ± 109 |
| | 10 | 10 | 176 ± 35‡ |
| 0.5% MC | – | 8 | 514 ± 150 |
| Maprotiline | 1 | 8 | 242 ± 49 |
| | 3 | 9 | 138 ± 24† |
| | 10 | 7 | 138 ± 24† |
| Saline | – | 11 | 393 ± 79 |
| Viloxazine | 1 | 10 | 562 ± 142 |
| | 3 | 11 | 264 ± 44 |
| | 10 | 10 | 119 ± 25‡ |
| 0.5% MC | – | 8 | 413 ± 70 |
| Citalopram | 1 | 8 | 319 ± 57 |
| | 3 | 9 | 392 ± 101 |
| | 10 | 8 | 296 ± 60 |
| Saline | – | 8 | 369 ± 128 |
| Alaproclate | 1 | 8 | 552 ± 191 |
| | 3 | 8 | 254 ± 49 |
| | 10 | 8 | 217 ± 82 |
| Saline | – | 8 | 369 ± 128 |
| Zimeldine | 1 | 8 | 262 ± 136 |
| | 3 | 8 | 514 ± 163 |
| | 10 | 8 | 480 ± 166 |

Each value represents mean ± SE. Each drug was administered IV to mice 10 min prior to the head injury. Each comparison was done by Mann-Whitney $U$-test when the Kruskal-Wallis $H$-test was significant ($p < 0.05$).

*Time from the physical insult to the onset of spontaneous movement (DT time).

Significantly different from the value for respective saline group: †$p < 0.05$, ‡$p < 0.01$.

TABLE 2

EFFECTS OF INDELOXAZINE HCl, ITS OPTICAL ISOMERS, AND SELECTIVE MONOAMINE UPTAKE INHIBITORS ON THE STEP-THROUGH PASSIVE AVOIDANCE RESPONSE IN RATS

| Treatment | Dose (mg/kg) | n | Latency of Step-Through (seconds) |
|---|---|---|---|
| Saline | – | 9 | 197 ± 56 |
| Indeloxazine | 1 IP | 8 | 386 ± 75 |
| | 2 | 8 | 463 ± 70* |
| | 4 | 8 | 291 ± 77 |
| | 8 | 8 | 362 ± 76 |
| Distilled water | – | 8 | 190 ± 29 |
| Indeloxazine | 3 PO | 8 | 201 ± 61 |
| | 10 | 8 | 440 ± 65† |
| | 30 | 8 | 271 ± 52 |
| Saline | – | 8 | 218 ± 66 |
| (−)-Indeloxazine | 1 IP | 8 | 364 ± 87 |
| | 2 | 8 | 474 ± 58* |
| | 4 | 8 | 169 ± 66 |
| | 8 | 8 | 129 ± 35 |
| Saline | – | 8 | 218 ± 66 |
| (+)-Indeloxazine | 1 IP | 8 | 263 ± 100 |
| | 2 | 8 | 442 ± 75* |
| | 4 | 8 | 203 ± 85 |
| | 8 | 8 | 223 ± 70 |
| 0.5% MC | – | 8 | 200 ± 52 |
| Citalopram | 1 IP | 8 | 315 ± 83 |
| | 3 | 8 | 288 ± 93 |
| | 10 | 8 | 399 ± 73* |
| Saline | – | 8 | 210 ± 58 |
| Alaproclate | 3 IP | 8 | 207 ± 61 |
| | 10 | 8 | 392 ± 59† |
| | 30 | 8 | 243 ± 79 |
| Saline | – | 8 | 203 ± 63 |
| Zimeldine | 3 IP | 8 | 195 ± 69 |
| | 10 | 8 | 366 ± 46* |
| | 30 | 8 | 221 ± 86 |
| 0.5% MC | – | 8 | 204 ± 88 |
| Maprotiline | 1 IP | 8 | 147 ± 70 |
| | 3 | 8 | 324 ± 87 |
| | 10 | 8 | 245 ± 85 |
| Saline | – | 8 | 228 ± 65 |
| Viloxazine | 3 IP | 8 | 222 ± 81 |
| | 10 | 8 | 209 ± 64 |
| | 30 | 8 | 267 ± 95 |
| Saline | – | 8 | 212 ± 63 |
| Amantadine | 3 IP | 8 | 209 ± 60 |
| | 10 | 8 | 251 ± 71 |
| | 30 | 8 | 201 ± 66 |
| Saline | – | 8 | 217 ± 54 |
| Amitriptyline | 1 IP | 8 | 173 ± 51 |
| | 2 | 9 | 193 ± 49 |
| | 4 | 8 | 56 ± 11* |

Each value represents mean ± SE. Each drug was administered IP and PO 60 and 30 min before training of passive avoidance task, respectively. Each comparison was done by the Mann–Whitney *U*-test.

Significantly different from the value for respective control group: *$p < 0.05$, †$p < 0.01$.

TABLE 3

EFFECTS OF INDELOXAZINE HCl, ITS OPTICAL ISOMERS, AND SELECTIVE MONOAMINE UPTAKE INHIBITORS ON THE STEP-THROUGH PASSIVE AVOIDANCE RESPONSE IN CYCLOHEXIMIDE-TREATED MICE

| Treatment | Dose (mg/kg) | Latency of Step-Through (seconds) |
|---|---|---|
| Distilled water | – | 48 ± 28 |
| Indeloxazine | 1 PO | 97 ± 36 |
| | 3 | 111 ± 36 |
| | 10 | 183 ± 39* |
| | 30 | 77 ± 29 |
| Distilled water | – | 37 ± 6 |
| (−)-Indeloxazine | 3 PO | 31 ± 4 |
| | 10 | 120 ± 22* |
| | 30 | 60 ± 22 |
| Distilled water | – | 37 ± 6 |
| (+)-Indeloxazine | 3 PO | 57 ± 17 |
| | 10 | 130 ± 31* |
| | 30 | 74 ± 15 |
| Distilled water | – | 43 ± 8 |
| Citalopram | 3 PO | 42 ± 22 |
| | 10 | 98 ± 20* |
| | 30 | 52 ± 28 |
| Distilled water | – | 38 ± 10 |
| Alaproclate | 3 PO | 44 ± 29 |
| | 10 | 126 ± 31* |
| | 30 | 57 ± 28 |
| Distilled water | – | 29 ± 12 |
| Zimeldine | 3 PO | 64 ± 28 |
| | 10 | 73 ± 27 |
| | 30 | 119 ± 36* |
| Distilled water | – | 43 ± 8 |
| Maprotiline | 3 PO | 40 ± 20 |
| | 10 | 54 ± 9 |
| | 30 | 50 ± 20 |
| Distilled water | – | 62 ± 28 |
| Viloxazine | 3 PO | 48 ± 21 |
| | 10 | 77 ± 38 |
| | 30 | 25 ± 13 |

Each value represents mean ± SE from 10 mice. Latency of step-through in normal mice was 236 ± 31 s ($n = 10$, mean ± SE). Cycloheximide in a dose of 150 mg/kg was administered sc 30 min before training. Test drugs were also administered orally 30 min before training. Each comparison was done by Wilcoxon's multiple comparison test when the Kruskal–Wallis *H*-test was significant ($p < 0.05$).

Significantly different from the value for respective control group: *$p < 0.05$.

## DISCUSSION

The present study demonstrated that indeloxazine and its optical isomers facilitated the spontaneous EEG in rats and ameliorated disturbances of consciousness in concussed mice. Mechanical head trauma often leads to somnolence, disturbance of consciousness, and synchronization of the EEG in humans (17). Therefore, these results suggested that indeloxazine and its optical isomers possessed arousal effects on the CNS. Indeloxazine and the (−)-isomer were about three times

TABLE 4

INHIBITORY EFFECTS OF INDELOXAZINE HCl AND ITS OPTICAL ISOMERS ON UPTAKE OF [$^{14}$C]NOREPINEPHRINE (NE) AND [$^{14}$C]5-HYDROXYTRYPTAMINE (5-HT) BY THE RAT BRAIN SYNAPTOSOMES

| | $IC_{50}$ ($\mu$M) | | |
|---|---|---|---|
| Treatment | 5-HT (whole brain) | NE (Hippocampus) | 5-HT/NE |
| Indeloxazine | 0.71 | 0.26 | 2.7 |
| (−)-Indeloxazine | 0.65 | 0.22 | 3.0 |
| (+)-Indeloxazine | 0.83 | 6.8 | 0.1 |

The $IC_{50}$ values, the concentrations of the test compounds to inhibit the uptake reaction by 50%, were obtained from the dose-response curves.

more potent than the (+)-isomer in arousal activity. In biochemical studies, the potencies of indeloxazine and the (−)-isomer in inhibiting NE uptake were approximately 25–30 times more potent than that of the (+)-isomer; however, the (+)-isomer inhibited 5-HT uptake with the same potency as indeloxazine and the (−)-isomer. Thus, the arousal spontaneous EEG seemed to involve an activation of the central noradrenergic system rather than the central serotonergic system. Shimizu-Sasamata et al. (19) reported that indeloxazine decreased the delta wave component of the spontaneous EEG mainly by facilitation of the central noradrenergic system in focal cerebral ischemic rats. In fact, it has been reported that the EEG desynchronizing actions of viloxazine, a selective NE uptake inhibitor, are related to stimulation of the central noradrenergic system (13). Further, indeloxazine and its optical isomers and selective NE uptake inhibitors possessed the arousal activity in concussed mice. On the other hand, selective 5-HT uptake inhibitors such as citalopram, alaproclate, and zimeldine showed little effect. The arousal activities of indeloxazine in the CNS may, therefore, be closely related to facilitation of the central noradrenergic system.

The relationship between the central serotonergic system and learning behavior using passive avoidance task was also studied. There are few comparative studies of the effects of selective monoamine uptake inhibitors on learning behavior. Indeloxazine and its optical isomers facilitated passive avoidance learning behavior in normal rats and ameliorated disturbed learning behavior in cycloheximide-treated mice. It has been reported that cycloheximide inhibits not only protein synthesis but also monoamine synthesis and release (5,6). These deficiencies are thought to disturb cognitive function in cycloheximide-treated rodents (16). Unlike arousal activity, indeloxazine and the (−)-isomer were equipotent to the (+)-isomer in their effects on learning behavior. In biochemical results, indeloxazine and the (−)-isomer were as potent as the (+)-isomer in inhibiting 5-HT uptake. Selective 5-HT uptake inhibitors such as citalopram, alaproclate, and zimeldine also facilitated and ameliorated learning acquisition in both non-treated rats and amnesic mice. In contrast, selective NE uptake inhibitors such as maprotiline and viloxazine and a selective DA uptake inhibitor such as amantadine had little effect on learning behavior. Amitriptyline, with both NE and 5-HT uptake inhibitory activities, deteriorates learning behavior in normal rats, presumably, in part, due to its anticholinergic activity. Data such as latencies during the acquisition trial and proportion of animals per treatment returned to the safe side by the 2-s shock administration could be useful in substantiating claims that the facilitatory effects are not due either to motor changes or changes in pain threshold. In our studies, these parameters were not influenced by administration of the test drugs. Therefore, the effects of the test drugs on learning behavior are due to the neurophysiological facilitation of cognitive function, not to either motor changes or changes in pain threshold.

These behavioral differences among monoamine uptake inhibitors may be attributable to their different selectivity for the central monoaminergic systems. Thus, enhancement of learning behavior seemed to involve an activation of the central serotonergic system rather than that of the central noradrenergic system. These results were supported by a previous report that indeloxazine ameliorated disturbed learning behavior mainly by facilitation of the central serotonergic system in focal ischemic rats (19). In the present study, indeloxazine and its optical isomers showed 5-HT uptake inhibition of similar potency to alaproclate and zimeldine (4). A number of reports indicate the involvement of brain 5-HT neurons in the processes of learning and memory in rodents (1–3,14,21). Alaproclate, for example, a selective 5-HT uptake inhibitor, enhances memory retrieval in mice (1). Moreover, patients with dementia disorders treated with citalopram showed a significant improvement in emotional disturbances (13). From the present and other results, it is suggested that the activating and ameliorating effects of indeloxazine on learning behavior may be ascribable to the accumulation of intrasynaptic 5-HT, which facilitates the activity of postsynaptic neurons (22) by inhibiting 5-HT reuptake.

In conclusion, the present results showed that indeloxazine and its optical isomers possess facilitatory effects on the CNS, and the pharmacological profiles of indeloxazine and its optical isomers with NE and 5-HT uptake inhibitory activities were wider than those of other selective monoamine uptake inhibitors and the classical tricyclic antidepressant (amitriptyline) tested in the present study. In addition, it is suggested that the central serotonergic and noradrenergic systems might be involved in learning behavior and central arousal action, respectively.

ACKNOWLEDGEMENTS

The authors are indebted to Drs. K. Murase and S. Usuda for their invaluable advice. They gratefully acknowledge the skillful technical assistance of Hiromi Takeuchi and the Astra Research Centre for supplying alaproclate and zimeldine.

## REFERENCES

1. Altman, H. J.; Nordy, D. A.; Ögren, S. O. Role of serotonin in memory: Facilitation by alaproclate and zimeldine. Psychopharmacology (Berl.) 84:496–502; 1984.
2. Altman, H. J.; Stone, W. S.; Ögren, S. O. Evidence for a possible functional interaction between serotonergic and cholinergic mechanisms in memory retrieval. Behav. Neural. Biol. 48:49–62; 1987.
3. Dehlin, O.; Hedenrud, B.; Jansson, P.; Nörgard, J. A double-blind comparison of alaproclate and placebo in the treatment of

patients with senile dementia. Acta Psychiatr. Scand. 71:190–196; 1985.
4. Elliott, J. M.; Stephenson, J. D. Depression. In: Webster, R. A.; Jordan, C. C., eds. Neurotransmitters, drugs and disease. Oxford: Blackwell Scientific Publications; 1989:355–393.
5. Flexner, L. B.; Goodman, R. H. Studies of memory: Inhibitors of protein synthesis also inhibit catecholamine synthesis. Proc. Natl. Acad. Sci. USA 72:4660–4663; 1975.
6. Freedman, L. S.; Judge, M. E.; Quartermain, D. Effects of cycloheximide, a protein synthesis inhibitor, on mouse brain catecholamine biochemistry. Pharmacol. Biochem. Behav. 17:187–191; 1982.
7. Harada, M.; Maeno, H. Biochemical characteristics of a potential antidepressant, 2-(7-indenyloxymethyl)morpholine hydrochloride (YM-08054-1). Biochem. Pharmacol. 28:2645–2651; 1979.
8. Jarbik, M. E.; Kopp, A. An improved one-trial passive avoidance learning situation. Psychol. Rep. 21:221–224; 1967.
9. Kojima, T.; Niigata, K.; Fujikura, T.; Tachikawa, S.; Nozaki, Y.; Kagami, S.; Takahashi, K. Syntheses of (+)-2-[(inden-7-yloxy)methyl] morpholine hydrochloride (YM-08054, indeloxazine hydrochloride) and its derivatives with potential cerebral-activating and antidepressive properties. Chem. Pharmacol. Bull. 33:3766–3744; 1985.
10. Lassen, J. B.; Squires, R. F.; Christensen, J. A.; Molander, L. Neurochemical and pharmacological studies on a new 5HT uptake inhibitor, FG4963, with potential antidepressant properties. Psychopharmacologia 42:21–26; 1975.
11. Manaka, S.; Sano, K. Effects of thyrotropin releasing hormone tartrate (TRH-T) on disturbance of consciousness following head injury in mice. Igaku Ayumi 102:867–869; 1977 (in Japanese).
12. Neal, H.; Bradley, P. B. Electrophysiological studies with a new antidepressant drug: Comparison of the effects of viloxazine (ICI58,834) with three tricyclic antidepressants in the encéphale isolé. Neuropharmacology 17:835–849; 1978.
13. Nyth, A. L.; Gottfries, C. G. The clinical efficacy of citalopram in treatment of emotional disturbances in dementia disorders: A Nordic multicentre study. Br. J. Psychiatry 157:894–901; 1990.
14. Ögren, S. O. Evidence for a role of brain serotonergic neurotransmission in avoidance learning. Acta Physiol. Scand. 544(suppl.): 1–71; 1985.
15. Ohtomo, E. Clinical evaluation of YM-08054 (indeloxazine) in the treatment of cerebrovascular disorder. Igaku Ayumi 136:535–555; 1986 (in Japanese).
16. Quinton, E. E. The cycloheximide-induced amnesia gradient of a passive avoidance task. Psychonom. Sci. 25:295–296; 1971.
17. Sano, K. Clinical studies on thyrotropin releasing hormone tartrate for the treatment of disturbance of consciousness. Adv. Neurol. Sci. 23:184–210; 1979 (in Japanese).
18. Schacht, U.; Heptner, W. Effects of nomifensine (Hoe 984), a new antidepressant, on uptake of noradrenaline in rat brain synaptosomes. Biochem. Pharmacol. 23:3413–3422; 1974.
19. Shimizu-Sasamata, M.; Yamamoto, M.; Okada, M.; Yamaguchi, T.; Tamura, A. Effects of indeloxazine hydrochloride on behavioral and biochemical changes in the chronic phase of focal cerebral ischemia in rats. Arch. Int. Pharmacodyn. 314:74–89; 1991.
20. Smith, D. F. The stereoselectivity of serotonin uptake in brain tissue and blood platelets: The topography of the serotonin uptake area. Neurosci. Biobehav. Rev. 10:37–46; 1986.
21. Weingartner, H.; Rudorfer, M.; Buchsbaum, M. S.; Linnoila, M. Effects of serotonin on memory impairments produced by ethanol. Science 221:472–474; 1983.
22. Yamaguchi, T.; Harada, M.; Nagano, H. Effects of indeloxazine hydrochloride (YM-08054) on monoamines and their metabolites in rat brain. Biogen. Amines 3:21–32; 1985.
23. Yamamoto, M.; Shimizu, M. Cerebral activating properties of indeloxazine hydrochloride. Neuropharmacology 26:761–770; 1987.
24. Yamamoto, M.; Shimizu, M. Effects of indeloxazine hydrochloride (YM-08054) on anoxia. Arch. Int. Pharmacodyn. 286:272–281; 1987.
25. Yamamoto, M.; Shimizu, M.; Sakamoto, N.; Ohtomo, H.; Kogure, K. Protective effects of indeloxazine hydrochloride on cerebral ischemia in animals. Arch. Int. Pharmacodyn. 290:16–24; 1987.