Pharmacology Biochemistry and Behavior, Vol. 45, pp. 445–450, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of Indeloxazine HCl on Kindled Amygdaloid Seizures in Rats: Comparison With the Effects of Phenytoin, Diazepam, Ethanol, and Imipramine

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Received 13 May 1992

NAKAMURA J., T. ANRAKU, M. SHIROUZU, Y. IWASHITA AND Y. NAKAZAWA. *Effects of indeloxazine HCl on kindled amygdaloid seizures in rats: Comparison with the effects of phenytoin, diazepam, ethanol, and imipramine.* PHARMACOL BIOCHEM BEHAV 45(2) 445–450, 1993.—The anticonvulsant effect of [(±)-2-[(inden-7-yloxy)methyl]morpholine (indeloxazine) HCl, a new cerebral activator, was investigated in rats against kindled seizures from the amygdala, an assumed model of secondarily generalized seizures in human. Indeloxazine (0.25–10 mg/kg, IP) dose-dependently depressed the kindled seizure and shortened the evoked amygdaloid afterdischarge. A high dose of indeloxazine (40 mg/kg, IP), however, induced generalized seizures. Comparison of the effects on the kindled seizures of indeloxazine to those of phenytoin, diazepam, ethanol, and imipramine revealed that the anticonvulsant actions of indeloxazine are similar to those of imipramine but not to those of phenytoin, ethanol, and diazepam. The results suggest that indeloxazine may exert its action through the monoaminergic system in the brain.

Indeloxazine HCl Cerebral activator Anticonvulsant Amygdala kindling Phenytoin Diazepam Ethanol Imipramine

SOME drugs that stimulate cerebral metabolism and increase cerebral blood flow act as anticonvulsants in addition to having antiamnestic and antihypoxic actions (5,12). The predominant use of acute animal models in epilepsy studies has been claimed to be responsible for discrepancies between experimental and clinical findings (21). Thus, in this study we used amygdaloid kindling in rats (1,2,8,13,19,23), a proposed model of secondarily generalized seizures in humans, to examine the anticonvulsant effect of [(±)-2-[(inden-7-yloxy)methyl]morpholine (indeloxazine) HCl (YM-08054) (22,24,25), a new cerebral activator, and compare it with that of phenytoin, diazepam, ethanol, and imipramine.

Indeloxazine has recently been introduced as a cerebral metabolic enhancer and shown to be effective in ameliorating psychiatric symptoms in patients with cerebral vascular diseases (9). The chemical structure of indeloxazine HCl is shown in Fig. 1.

METHOD

Forty-four Wistar male rats (250–350 g at surgery) were used. Animals were housed with free access to food and water under a 12 L : 12 D cycle (light 0600–1800 h). Using a stereotaxic apparatus under sodium pentobarbital anesthesia (50 mg/kg, IP), a bipolar electrode (tungsten wires, 0.15 mm in diameter) was inserted into the left amygdala (P = 2.8 mm, L = 5.0 mm, H = 7.8 mm) for stimulation and chronic recording. A reference recording electrode was inserted into the right amygdala according to the atlas by Paxinos and Watson (16). A screw was attached to the occipital bone for reference. Electrodes and screw were fixed to the skull with dental cement.

A stimulator (Nihon Koden:SEN 3201) was connected to an isolator. EEG recordings were made with an electroencephalograph (SAN-EI:1A-71). An automated switching box was

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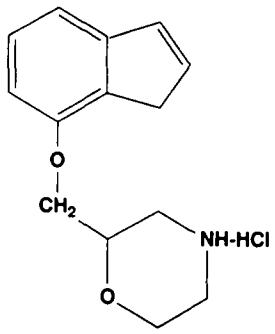

FIG. 1. Chemical structure of indeloxazine HCl.

used to disconnect the EEG during a 1-s stimulus and reconnect it after the stimulus.

The afterdischarge (AD) threshold was determined for each rat as described by Freeman and Jarvis (7). Starting at 25 μA, the amygdala was stimulated at intervals of not less than 5 min, with increments of 25 μA. The AD threshold was defined as the stimulation intensity producing AD spikes in the EEG and/or behavioral signs such as brief immobility, eye closure, or mouth clonus. The range of AD thresholds was 75–225 μA.

Starting 1 day after AD threshold determination, a 1-s train of 60 Hz, 1-ms square waves was delivered to each rat three times a day at intervals of at least 3 h. The AD duration and amplitude, behavioral seizure duration, and stage of seizure were recorded. Severity of seizure (seizure stage) was ranked according to Racine (17). Rats were considered kindled when they developed at least three consecutive stage-5 seizures. The threshold for kindling is referred to as the final electrical threshold (FET).

Three experimental protocols were followed.

Experiment 1: Acute Anticonvulsant Effect of Indeloxazine

Indeloxazine dissolved in saline was administered IP at doses of 0.25, 0.5, 1, and 10 mg/kg. One hour after administration, the amygdala was stimulated at the FET determined the previous day. Elicited seizure stage and AD duration were compared with those observed after administration of saline the previous day.

Experiment 2: Time Course of Anticonvulsant Effect of Indeloxazine

Indeloxazine, 1 and 10 mg/kg, was administered IP to 15 rats with established amygdaloid kindling and its anticonvulsant effect determined 30 min, 1, 2, 4, 6, and 8 h after administration. Only one interval was tested per day in a given animal.

Experiment 3: Anticonvulsant Effect of Phenytoin, Diazepam, Ethanol, and Imipramine

A dose of 20, 40, or 60 mg/kg phenytoin, 2 mg/kg diazepam, 1 g/kg ethanol, or 10 mg/kg imipramine was administered IP to established amygdaloid-kindled rats and the anticonvulsant effects of these compounds examined 1 h later as in Experiment 1 above.

In all these experiments, the washout period was set to over 3 days for indeloxazine and over 5 days for the other drugs and the order of drug administration at given time points randomized for individual rats. The drugs used were indeloxazine HCl (Yamanouchi), phenytoin (Dainippon), diazepam (Takeda), ethanol (Wako), and imipramine (Ciba-Geigy). All drugs were delivered IP in a volume of 1 ml/kg body wt., with diazepam given as suspensions in 4 N HCl and saline. Indeloxazine HCl, phenytoin, and imipramine were dissolved in 0.9% NaCl (saline). Ethanol (20% v/v) was prepared by mixing 99.5% ethanol with saline. After the experiments, animals were sacrificed and localization of the electrodes in the brain was verified histologically. Parametric data were analyzed statistically using Student's paired *t*-test. Nonparametric data (severity of seizure) were analyzed with the Wilcoxon signed-rank test. A value of $p < 0.05$ was considered the criterion for significance.

RESULTS

Experiment 1: Acute Anticonvulsant Effect of Indeloxazine

One hour after IP administration of indeloxazine, both the kindled seizure convulsion stage and the AD duration de-

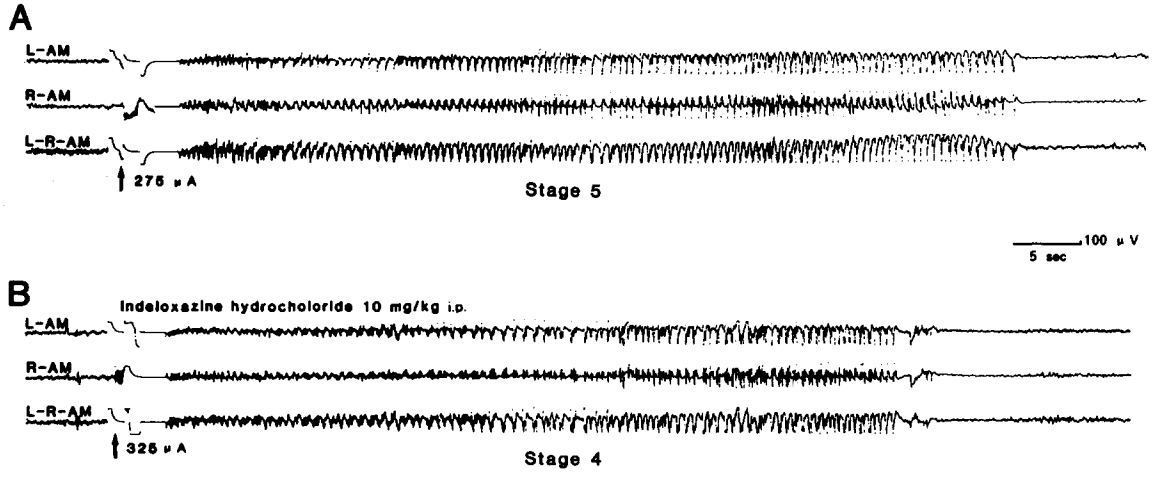

FIG. 2. (A) Stage-5 seizure elicited at the final electrical threshold (FET), 275 μA. (B) Thirty minutes after 10 mg/kg indeloxazine HCl, stage 4 was elicited by 325 μA, that is, 50 μA over the FET.

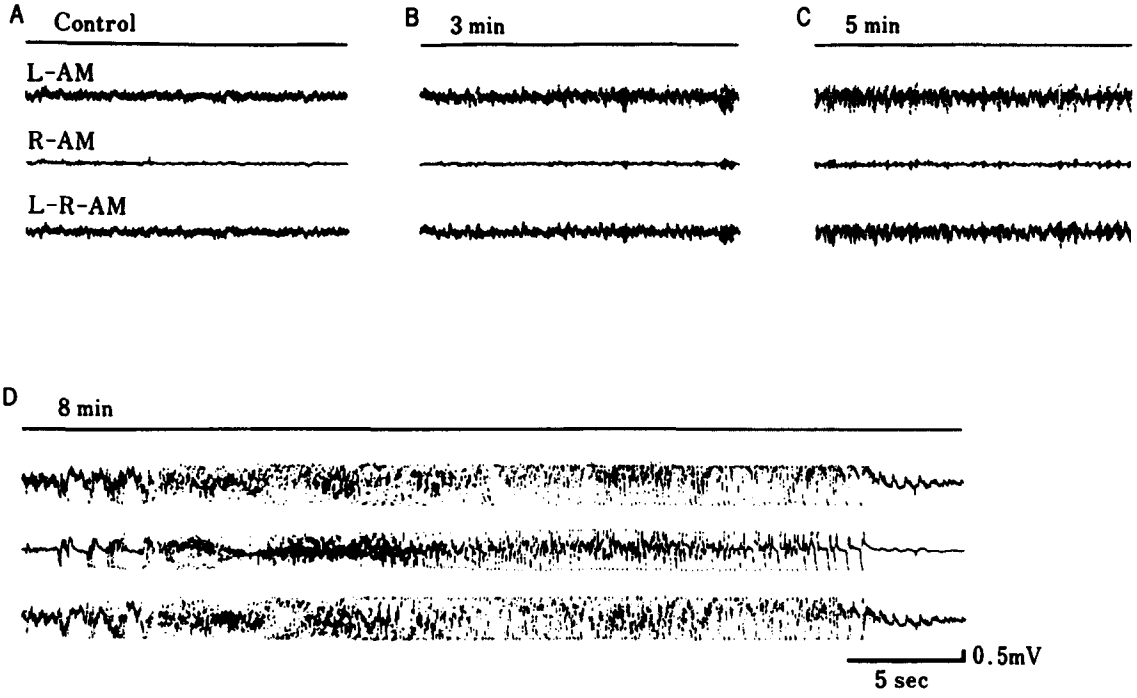

FIG. 3. Effects of indeloxazine (40 mg/kg, IP) on the pattern of EEG. (A) Control. (B) Three minutes after injection of indeloxazine. (C) Five minutes after injection. (D) Eight minutes after injection. The rat showed ataxia in (C) and tonic-clonic generalized seizure in (D).

creased in a dose-dependent manner. Stage-5 kindled seizures developed in six of six rats after the 0.25-mg/kg dose and in two of six rats in the 0.5-mg/kg group. Rats receiving 10 mg/kg (IP) indeloxazine demonstrated no kindled seizures. When stimulus intensity was raised from 25 to 100 μA, stage-2 to stage-5 kindled seizures occurred in all six rats receiving 10 mg/kg (IP) indeloxazine (Fig. 2). No behavioral change was noted in rats receiving 0.25–10 mg/kg indeloxazine, but those receiving 20 mg/kg showed hypomyotonia and ataxia. Those receiving 40 mg/kg showed hypomyotonia and ataxia, followed by spontaneous convulsions (Fig. 3).

Experiment 2: Time Course of Indeloxazine Administration

The time course of the anticonvulsant effect of indeloxazine (10 mg/kg, IP) was examined. The anticonvulsant effect appeared 30 min after indeloxazine administration and peaked after 1 h.

Experiment 3: Anticonvulsant Effects of Phenytoin, Diazepam, Ethanol, and Imipramine

One hour after phenytoin (IP) administration at a dose of 20, 40, or 60 mg/kg, kindling stage and AD duration decreased. All six rats in the 20-mg/kg group and three of six rats in the 40-mg/kg group showed stage-5 convulsions, with two rats in the latter group showing no convulsions. Rats receiving 60 mg/kg phenytoin developed no convulsions.

One hour after 1 g/kg (IP) of ethanol administration, all six rats developed stage-2 convulsions.

When 10 mg/kg imipramine was IP administered, AD duration decreased to 1 h in all rats tested but systemic convul-

TABLE 1

INDELOXAZINE EFFECT ON KINDLED SEIZURE FROM RAT AMYGDALOID COMPLEX

AD Duration (seconds)			Seizure Stage	
Control	Ideloxazine HCl			
117.1 ± 6.6 (n = 6)	0.25 mg/kg	108.5 ± 15.6	5.0	(5–5)
110.9 ± 21.7 (n = 6)	0.5 mg/kg	38.5 ± 23.3*	2.0 ± 1.0	(0–5)
117.0 ± 7.1 (n = 7)	1 mg/kg	33.1 ± 21.6*	1.4 ± 0.9*	(0–5)*
113.1 ± 4.9 (n = 10)	10 mg/kg	0†	0‡	(0–0)

AD duration: Mean ± SEM. Seizure stage: Mean ± SEM. The numbers in parentheses indicate the seizure stages classified according to Racine. Two-tailed Wilcoxon's test was used to compare results between drug- and saline-treated rats.

*$p < 0.05$.

†$p < 0.01$.

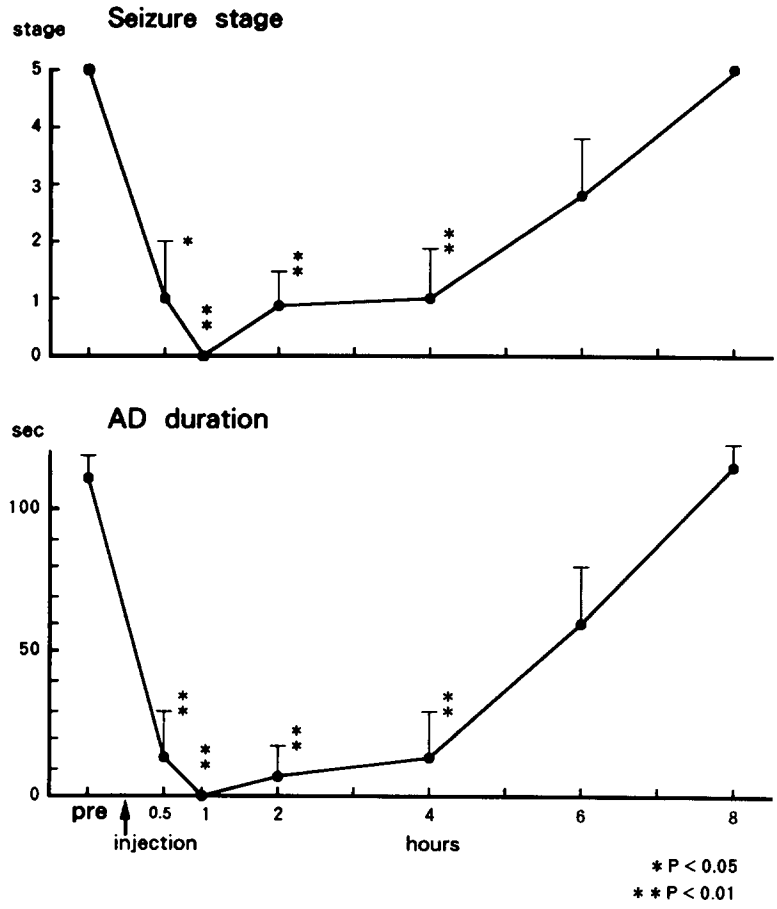

FIG. 4. Time course of anticonvulsant effect of indeloxazine on kindled seizure in rats (n = 15). Note that both afterdischarge (AD) duration and seizure stages were significantly decreased between 30 min and 4 h after injection of 10 mg/kg indeloxazine. Values: mean ± SEM.

sions were not suppressed. Although no behavioral change was induced by 10-mg/kg imipramine administration, hypomytonia and ataxia followed by spontaneous generalized seizures occurred in rats receiving 40 mg/kg imipramine.

Moreover, even when 60 mg/kg phenytoin, 1 g/kg ethanol, or 2 mg/kg diazepam showed anticonvulsant effects on kindled seizures, stage-5 convulsions were induced in all rats receiving phenytoin by increasing stimulus intensity from 25 to

TABLE 2

ANTICONVULSANT EFFECT ON THE FET FOR KINDLED SEIZURES IN RATS

	FET			
	+25	+50	+75	+100 μA
Indeloxazine 10 mg/kg (n = 6)	(2) (5)	(3)	(3)	(4) (5)
Phenytoin 60 mg/kg (n = 6)	(5) (5)	(5) (5) (5) (5)		
Diazepam 2 mg/kg (n = 5)	(2) (2) (2) (2) (3)			
Ethanol 1 g/kg (n = 6)	(2) (2) (2) (2) (3)	(3)		

Numbers in parentheses indicate the seizure stages (stages 1–5) in individual rats according to Racine.

50 μA. This increase from 25 to 50 μA caused stage-2 and -3 convulsions in all rats receiving diazepam and ethanol, respectively.

DISCUSSION

In this study, indeloxazine showed a biphasic action on amygdaloid kindling in rats, that is, anticonvulsant effects at doses ranging from 0.5–10 mg/kg, hypomyotonia and ataxia at doses of 20 and 30 mg/kg, and spontaneous generalized seizure at a dose of 40 mg/kg. Several studies reported that kindled seizures are suppressed by activation of noradrenergic neurons or by an increase in noradrenaline (NA) level in the CNS (4,10,11). Therefore, NAergic neurotransmitters may participate in the kindling suppression induced by indeloxazine. Like other anticonvulsants, stage-2 to -5 convulsions were induced in most rats when stimulus intensity was raised, while the effective anticonvulsant effect of indeloxazine was maintained. This indicates that the anticonvulsant effect of indeloxazine may be due to an increase in FET at the locus of stimulation. However, although phenytoin showed dose-dependent anticonvulsant effects in amygdaloid kindling increases in stimulus intensity induced only stage-5 convulsion. From these effects of phenytoin, it appears that phenytoin is therapeutically effective in epilepsy by suppressing the propagation of convulsions (23). In the cases of diazepam and ethanol, an increase in stimulus intensity induced convulsions not exceeding stage 2 and 3. Thus, a rise in FET at the locus of stimulation is suggested as the mechanisms of the anticonvulsant action of diazepam and ethanol. Diazepam and ethanol both potentiate the action of GABA, an inhibitory neurotransmitter in the CNS (3,6,18,19). Although it seems that indeloxazine shows diazepam- and/or ethanol-like anticonvulsant effects rather than a phenytoin-like effect, little is known about the effect of indeloxazine on GABA. Considering the anticonvulsant effect of indeloxazine, taking reported pharmacological and biochemical findings into account, it is more likely that indeloxazine increases the level of monoamines, such as NA, dopamine (DA), and serotonin (5-HT) (22,24,25), at the epileptic focus, thus shifting the level of FET up. Moreover, IP administration of 40 mg/kg indeloxazine induced generalized seizures instead of its anticonvulsant action. These effects of indeloxazine rather resemble the biphasic action of imipramine or nomifensine (20), catecholamine uptake inhibitors, on kindling. Imipramine also showed biphasic actions, namely, 10 mg/kg imipramine tended to shorten AD duration and 40 mg/kg imipramine caused tonic-clonic convulsion. It appears, therefore, that a small dose of imipramine releases catecholamines, which may cause the anticonvulsant effect, while a large dose blocks the release of catecholamines, inducing convulsions. From these results, it appears that the biphasic anticonvulsant effects of indeloxazine are similar to those of the anticonvulsant drugs. Calcium hopantenate (HOPA-Ca), one such drug for the treatment of cerebrovascular disorders that has been reported to have an anticonvulsant effect on the acute epilepsy rat model, was also shown to facilitate amygdaloid kindling (21). Moreover, clinical cases in which convulsions were induced by HOPA-Ca have been reported (14,15). In contrast, indeloxazine showed biphasic actions, that is, an anticonvulsant effect on amygdala-kindled seizures at a certain dose range and an induction of spontaneous generalized seizure at a larger dose. These findings suggest that this agent may have a clinical anticonvulsant effect. When metabolism and excretion are altered by aging and complications and/or the level of cerebral monoamines decrease in epileptic patients, indeloxazine might exert an anticonvulsant effect.

ACKNOWLEDGEMENTS

This article was kindly revised by Prof. Canio Polosa, Department of Physiology, McGill University, Canada. The authors are grateful to Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan), for supplying indeloxazine HCl.

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