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The involvement of monoaminergic and GABAergic systems in locomotor inhibition produced by clobazam and diazepam in rats

M. T. Hsieh

Department of Pharmacology, Tokyo Medical College, Shinjuku, Tokyo 160, Japan

Abstract. The effects of 1,5-benzodiazepine clobazam and diazepam were evaluated with regard to behavioral locomotor changes in rats. A concurrent focus of investigation was whether or not both benzodiazepines interact with central monoaminergic and GABAergic mechanisms. Diazepam was more active than clobazam in reducing locomotor activity. The stimulation of locomotor activity induced by L-dopa plus benserazide, and methamphetamine was significantly counteracted by diazepam but unaffected by clobazam. Hypomotility produced by α-methyl-p-tyrosine was markedly augmented with both drugs. Locomotor suppression elicited by 5-HTP, activating central serotoninergic transmission, was more potently reversed by clobazam than by diazepam. In addition, arousal behavior produced by p-CPA, inactivating central serotoninergic transmission, was completely abolished by both drugs. Furthermore, in combination with AOAA, known to inhibit motor activity, diazepam had a synergistic effect, and picrotoxin-produced suppression was significantly antagonized by diazepam. Both benzodiazepines thus may exert their behavioral depressant effects by reducing catecholaminergic and serotoninergic activity, and also by promoting GABA-mediated inhibition in the central nervous system. Clobazam is more effective than diazepam in reducing serotoninergic activity, but less effective in reducing catecholaminergic activity and increasing GABAergic activity.

Key Words: clobazam – diazepam – locomotor activity – monoamines – GABA

Numerous investigations on the mode of action of benzodiazepines indicate that the anxiolytic activity of this class of drugs is mediated by the central nervous system. Recent studies reveal that retarded synthesis and turnover of brain 5-HT [Rastogi et al. 1977] are possibly involved in the anxiolytic action of benzodiazepines. In this connection, Stein et al. [1977] and Haefely [1978] suggested that the sedative and anxiety-reducing effects of benzodiazepines might be due to changes in monoaminergic systems secondary to altered GABAergic transmission.

Clobazam (1-phenyl-5-methyl-8-chloro-1,2,4,5-tetra-hydro-2,4-diketo - 3H - 1,5 - benzodiazepine, Hoechst Japan) (Fig. 1) is a new 1,5-benzodiazepine derivative. The structure of this compound differs from that of conventional minor tranquilizers, since the nitrogen atoms in the heterocyclic ring are in the 1,5-instead of the 1,4-position. Pharmacologically, the anticonvulsant effect of clobazam was comparable to that of diazepam [Barzaghi et al. 1973], but clobazam was less active in Geller conflict in the rat [Fielding and Hoffmann 1979] and in inhibiting polysynaptic spinal reflexes in the rabbit [Gerhards 1978]. Whereas clobazam had a prolonged anxiolytic action in certain neuroses in a double-blind study with diazepam [Doongaji et al. 1979], it had minimal effects on human psychomotor performance [Berry et al. 1974]. However, effects of clobazam on the central nervous system remain obscure. This study was therefore carried out to investigate the effects of clobazam compared with diazepam and to determine if these drugs differ in their action on central monoaminergic and GABAergic systems affecting motor performance in the rat.

Reprint requests to Dr. M. T. Hsieh.

Materials and methods

Animals

Male Sprague-Dawley rats, weighing between 195 to 230 g and obtained from Nippon Charles River, were used throughout these studies. The animals were maintained in groups of five per cage at a controlled temperature of 22 ± 1° C and humidity of 55 ± 5%, and housed in a metal-wire mesh cage for at least 7 days before each experiment. A solid diet MF (Oriental Yeast Co., Tokyo) and tap water were provided ad libitum.

Fig. 1 The chemical structures of clobazam (CBZ) and diazepam (DZP).

Drugs and treatment regimens

The following compounds were used in these experiments: clobazam (CBZ, Hoechst Japan), diazepam (DZP, Hoffmann-La Roche), methamphetamine HCl (MAPT, Dainippon Pharmaceuticals), L-dopa (Kyowa Hakko Kogyo Co., Japan), benserazide HCl (Hoffmann-La Roche), α-methyl-p-tyrosine methylester HCl (α-MT, Sigma), 5-hydroxy-L-tryptophan (5-HTP, Kyowa Hakko Kogyo Co., Japan), P-chlorophenylalanine (P-CPA, Sigma), aminooxy acetic acid hemihydrochloride (AOAA, Sigma), and picrotoxin.

Clobazam or diazepam (1 and 10 mg/kg i.p.) were administered in a fixed dosage volume of 2 ml/kg body weight. MAPT and picrotoxin were given s.c. into the dorsal part of the neck in a volume of 1 ml/kg. Except for concomitant drug treatment, all other compounds were injected intraperitoneally in a volume of 2.5 ml/kg. Whereas 5-HTP (50 mg/kg) and clobazam or diazepam were administered concomitantly to one rat, they were injected several seconds apart into different intraperitoneal sites. The other compounds were given before clobazam or diazepam as follows: α-MT (100 mg/kg) every 2 h, p-CPA (200 mg/kg) every 24 h, L-dopa (200 mg/kg) every 50 min, benserazide (50 mg/kg) every 80 min, AOAA (12.5 mg/kg) every 3 h, picrotoxin (0.25 mg/kg) every 10 min. Control rats received injection of the appropriate vehicle, 0.5% CMC. Clobazam, diazepam, L-dopa, 5-HTP, and p-CPA were suspended in 0.5% CMC solution; all the other compounds were dissolved in purified water. L-Dopa, 5-HTP, AOAA, and picrotoxin were prepared immediately before use.

Locomotor activity measurements

The locomotor activity of individual rats was measured with an Animex Activity Meter Type S, and an Animex Counter Type 1–6 (Farad Electronics, Hägersten, Sweden). During the course of the experiments the Animex Activity Meter was placed in a sound-proofed, temperature-controlled, poorly illuminated room used solely for this experiment. After treatment rats were immediately placed in individual opaque plastic cages having dimensions of 40 × 30 × 45 cm. The sensitivity and tuning of the instrument was then adjusted to 35 μA to enable all kinds of motor behavior, including locomotion, rearing, grooming, sniffing, and licking to be jointly recorded. Activity was recorded for 60-min intervals for 3 h, starting 5 min after inserting the animal into the test cage. Each rat was used only once.

Statistical analysis

Results were analyzed statistically using Student's t test to compare intergroup differences.

Results

The effects of intraperitoneal injections of clobazam and diazepam on locomotor activity

Table 1 shows the results of an experiment in which various doses of clobazam (CBZ) and diazepam (DZP) were administered to groups of 10 rats. The lower dose of clobazam (1 mg/kg) had no statistically significant effect on locomotor activity ($p > 0.05$), whereas the higher dose of 10 mg/kg significantly decreased activity to 417 ± 45 ($p < 0.001$) from control values of 892 ± 80. In contrast to clobazam, diazepam at both doses (1 and 10 mg/kg) significantly decreased locomotor activity to 497 ± 54 ($p < 0.01$) and 401 ± 41 ($p < 0.001$), respectively. Sedative behavior and decreased ambulation were also seen.

The effects of clobazam and diazepam on methamphetamine-produced locomotor activity

The results are shown in Fig. 2. MAPT alone in a dose of 0.1 mg/kg produced an increase in activity, but the degree was not significantly different from that with 0.5% CMC ($p > 0.1$). The locomotor activity produced by MAPT alone was markedly reduced by concomitant administration of diazepam (10 mg/kg) ($p < 0.01$), but not by clobazam (1 and 10 mg/kg).

Table 1 Effects of CBZ and DZP on locomotor activity in rats.

Treatment (mg/kg)	No. of rats	Locomotor activity (counts/ 60 min)	Probability[b]
Control	24	892 ± 80[a]	
CBZ 1 mg/kg	10	714 ± 73	N.S.
CBZ 10 mg/kg	10	417 ± 45	$p < 0.001$
DZP 1 mg/kg	10	497 ± 54	$p < 0.01$
DZP 10 mg/kg	10	401 ± 41	$p < 0.001$

[a] Each value represents the mean ± S.E. of 10 or 24 rats per group. Rats were injected i.p. with either CBZ or DZP. Control rats were injected with an equal volume of the vehicle, 0.5% CMC. Locomotor activity was recorded for 60 min, starting 5 min after inserting the rat into the test cage.

[b] Probability was calculated using Student's t test. Statistically significant differences are relative to the values of control rats. N.S. denotes non-significant.

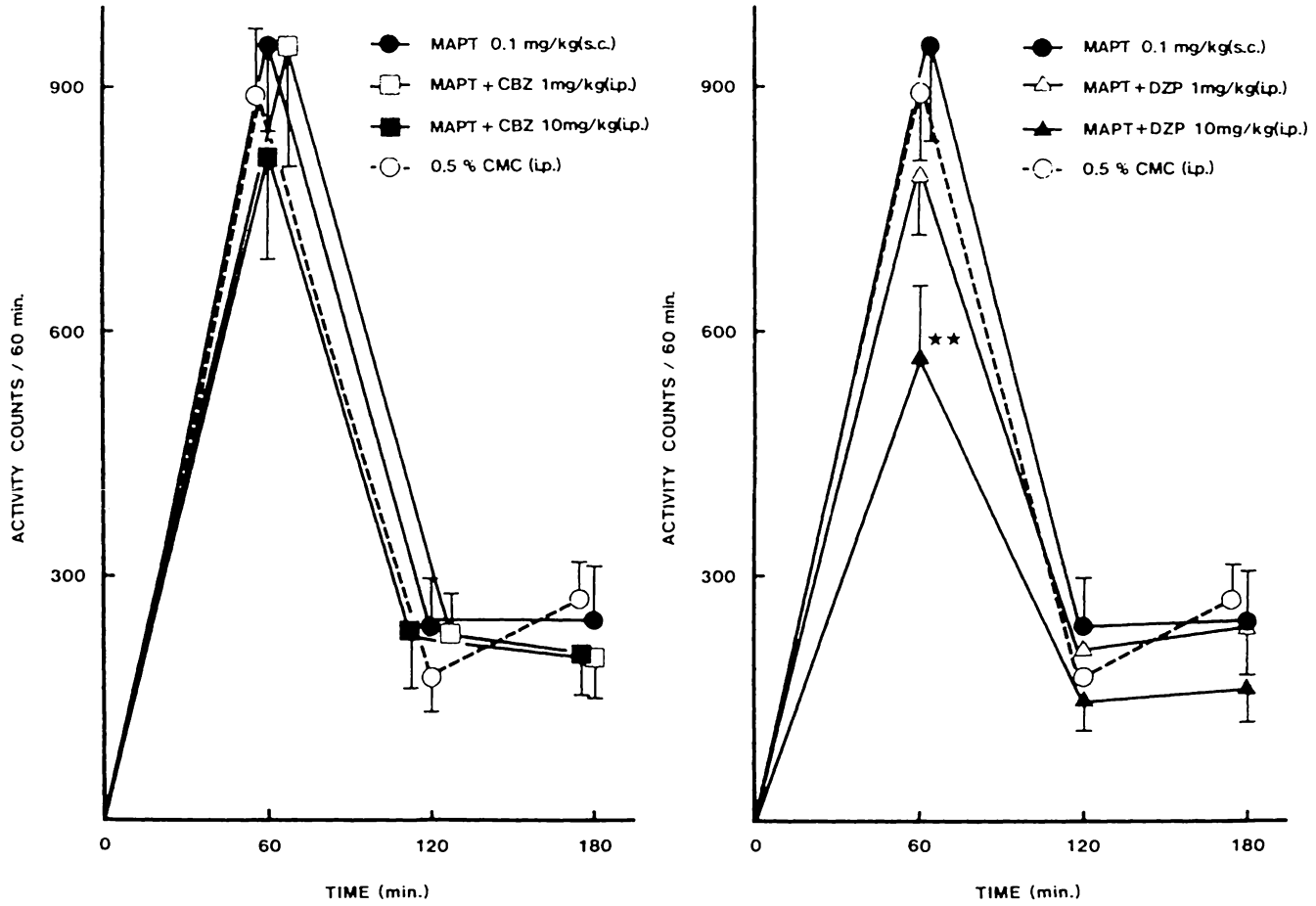

Fig. 2 Effects of CBZ and DZP on changes in locomotor activity produced by MAPT. An intraperitoneal injection of CBZ or DZP was given simultaneously with MAPT (0.1 mg/kg) s.c. Locomotor activity was measured at 60-min intervals for 3 h, starting 5 min after inserting the rat into the test cage as described. In all experiments, each point represents the mean obtained from 10 animals except for the 0.5% CMC group (24 animals). Vertical bars indicate s.e.m. The asterisks indicate values which are significantly different from the corresponding results for MAPT (0.1 mg/kg) administration (** $p < 0.01$).

The effects of clobazam and diazepam on L-dopa plus benserazide-produced locomotor stimulation

Rats treated with L-dopa exhibited a slight but significant decrease in locomotor activity (409 ± 85, $p < 0.05$). The combination of L-dopa and benserazide caused a greater increase in locomotor activity ($p < 0.05$ to $p < 0.001$) (Fig. 3), commencing about 30 min after injection and continuing for at least 3 h until the animals were exhausted. In addition to this locomotor stimulation, stereotyped movements, including head shaking, forepaw padding, jumping, sniffing, and licking, were observed. Autonomic symptoms, namely salivation, exophthalmos, and piloerection, were also seen.

As shown in Fig. 3, clobazam (1 mg/kg) slightly but not significantly increased the intensity of locomotor stimulation at 60 to 120 min. On the other hand, clobazam (10 mg/kg) and diazepam (1 mg/kg) slightly but not significantly decreased the response; but locomotor stimulation was strongly reduced by diazepam (10 mg/kg) ($p < 0.001$) at 60 to 180 min (Fig. 3).

The effects of clobazam and diazepam on α-MT-produced hypomotility

Rats pretreated with α-MT appeared to be behaviorally depressed ($p < 0.1$) as compared with the 0.5% CMC control (Fig. 4). As shown in Fig. 4, the hypomotility produced by α-MT was not affected by clobazam (1 mg/kg), but markedly augmented by clobazam (10 mg/kg) and diazepam (1 and 10 mg/kg) ($p < 0.01$), as compared with α-MT alone.

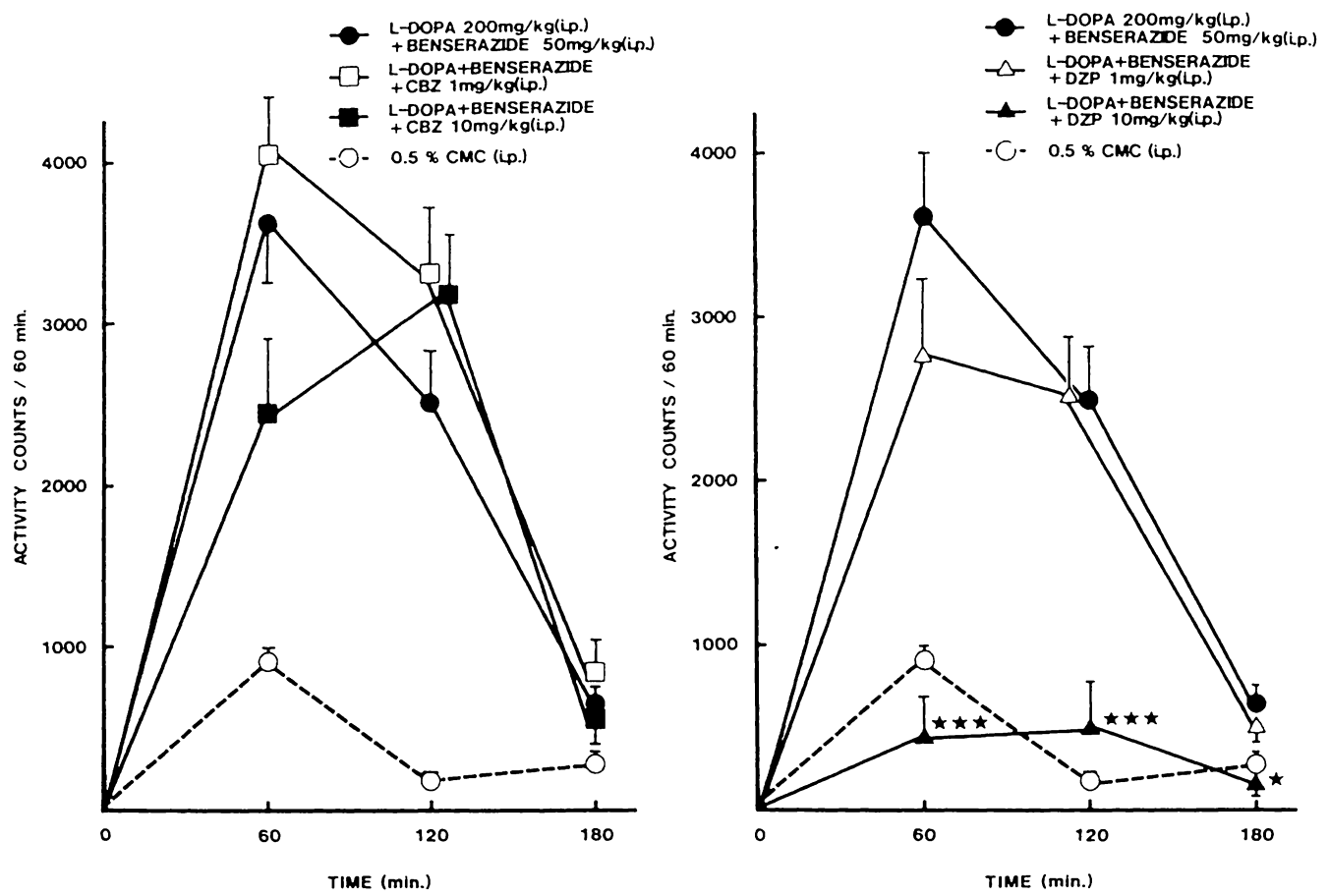

Fig. 3 Effects of CBZ and DZP on changes in locomotor activity produced by L-dopa plus benserazide. Benserazide (50 mg/kg) was injected 80 min and L-dopa (200 mg/kg) 50 min before CBZ or DZP. All drugs were injected i.p. For details see legend for Fig. 2. The asterisks indicate values that are significantly different from the corresponding results for L-dopa plus benserazide administration (* $p < 0.05$, *** $p < 0.001$).

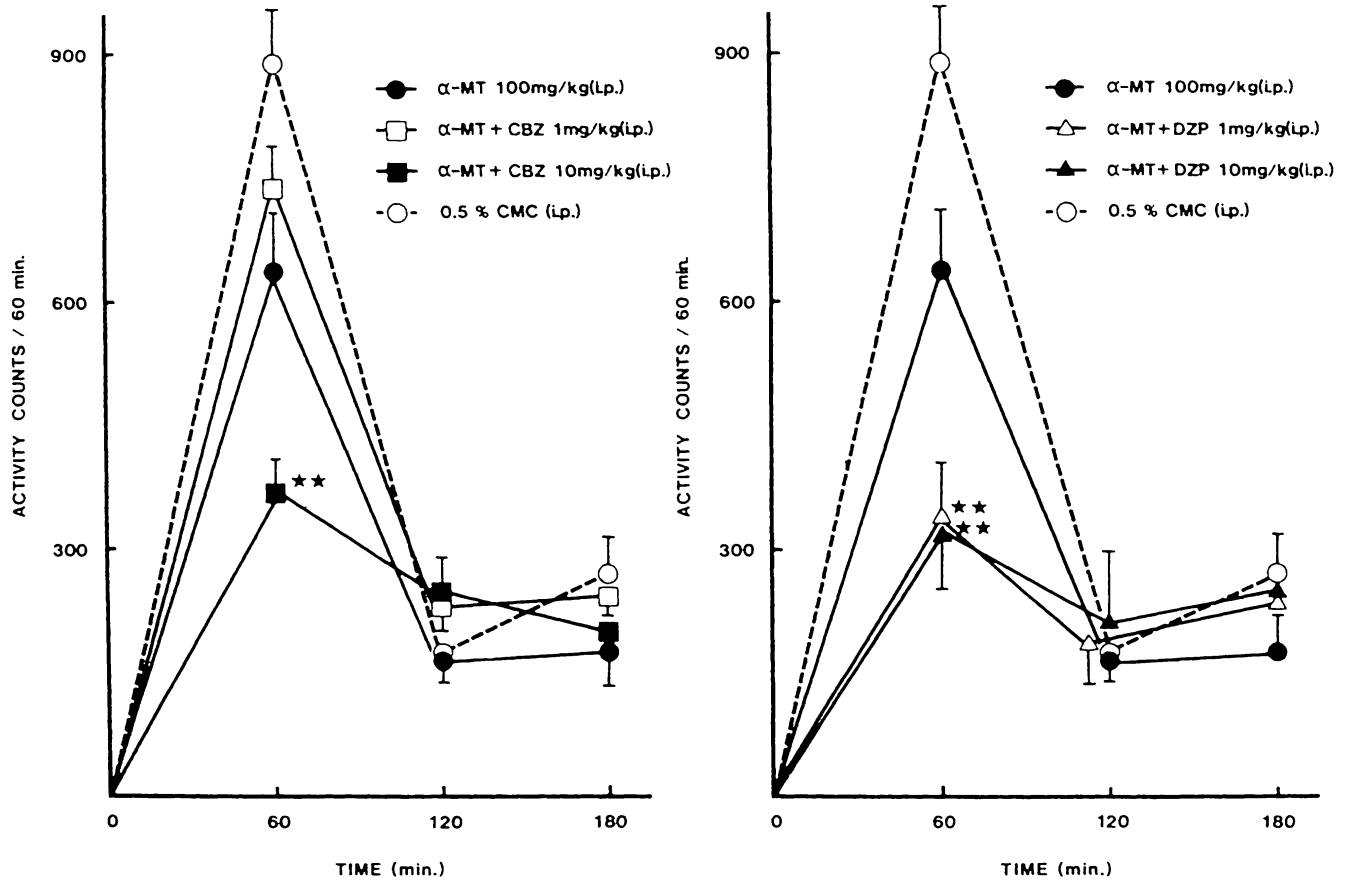

Fig. 4 Effects of CBZ and DZP on changes in locomotor activity produced by α-MT. α-MT was given in a dose of 100 mg/kg 2 h before CBZ or DZP. All drugs were injected i.p. For details see legend for Fig. 2. The asterisks indicate values that are significantly different from the corresponding results for α-MT administration (** $p < 0.01$).

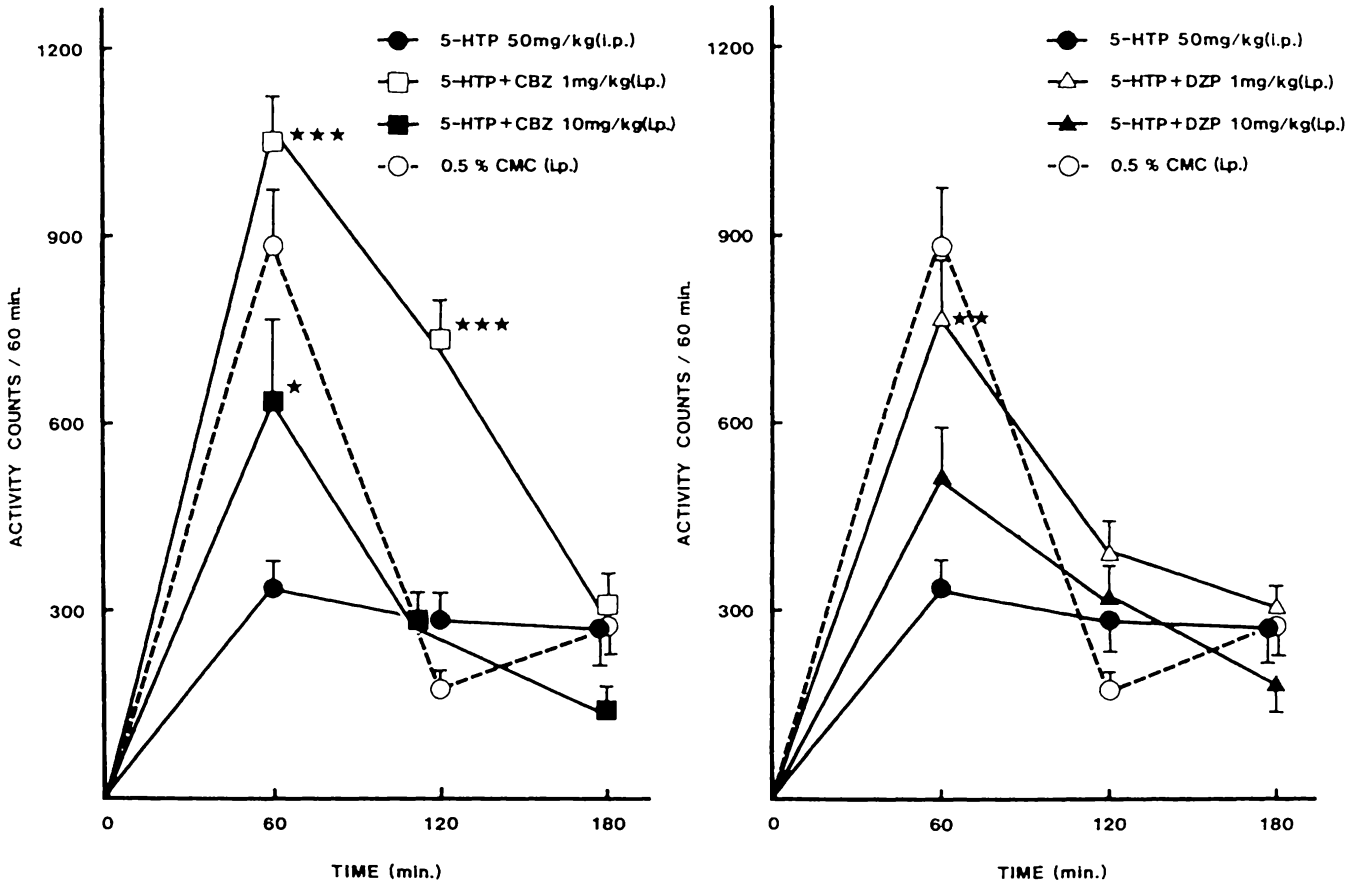

Fig. 5 Effects of CBZ and DZP on changes in locomotor activity produced by 5-HTP. CBZ or DZP was given simultaneously with 5-HTP (50 mg/kg). All drugs were injected i.p. For details see legend for Fig. 2. The asterisks indicate values that are significantly different from the corresponding results for 5-HTP administration (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

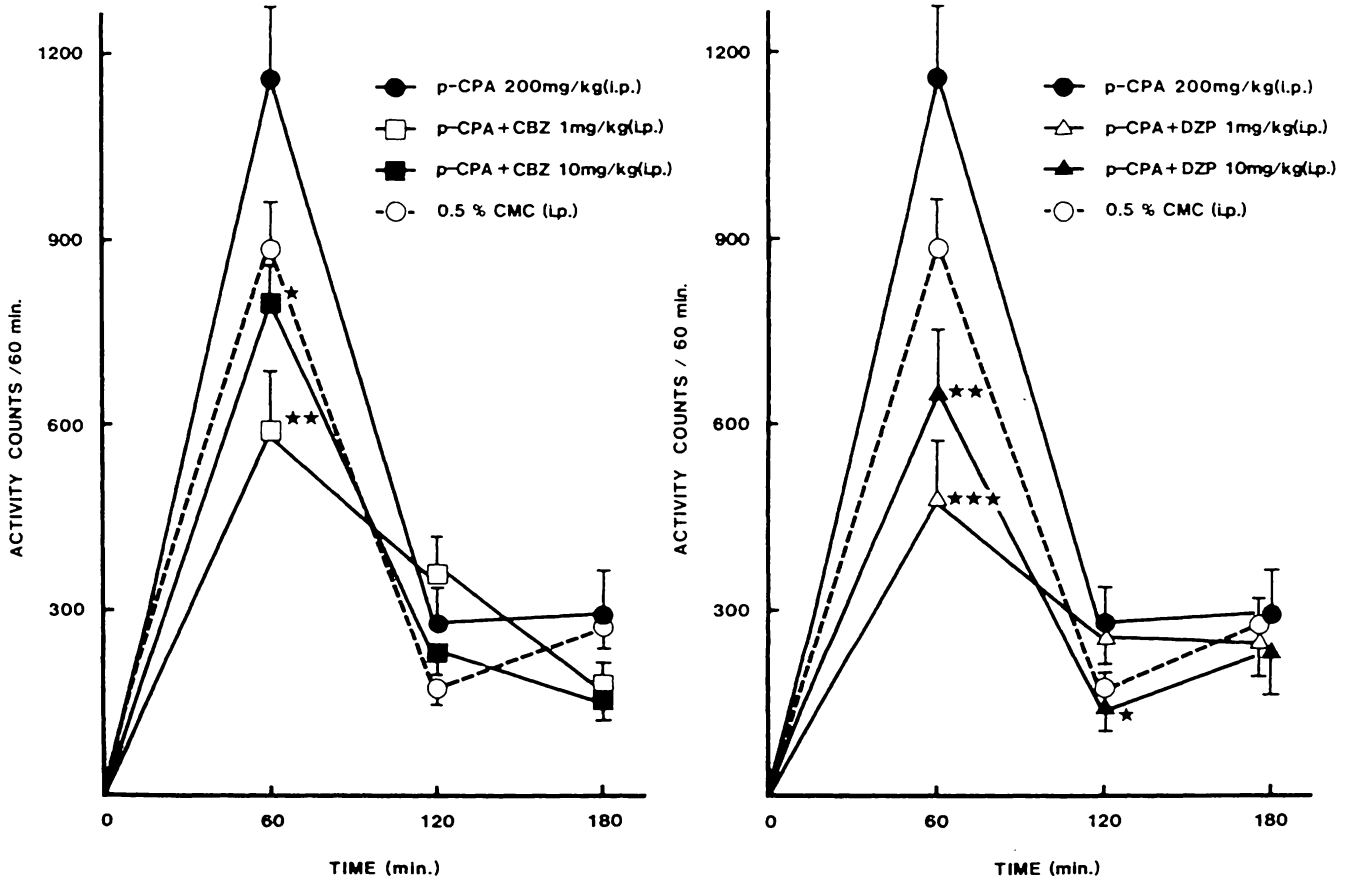

Fig. 6 Effects of CBZ and DZP on changes in locomotor activity produced by p-CPA. p-CPA was given in a dose of 200 mg/kg 24 h before CBZ or DZP. All drugs were injected i.p. For details see legend for Fig. 2. The asterisks indicate values that are significantly different from the corresponding results for p-CPA administration (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

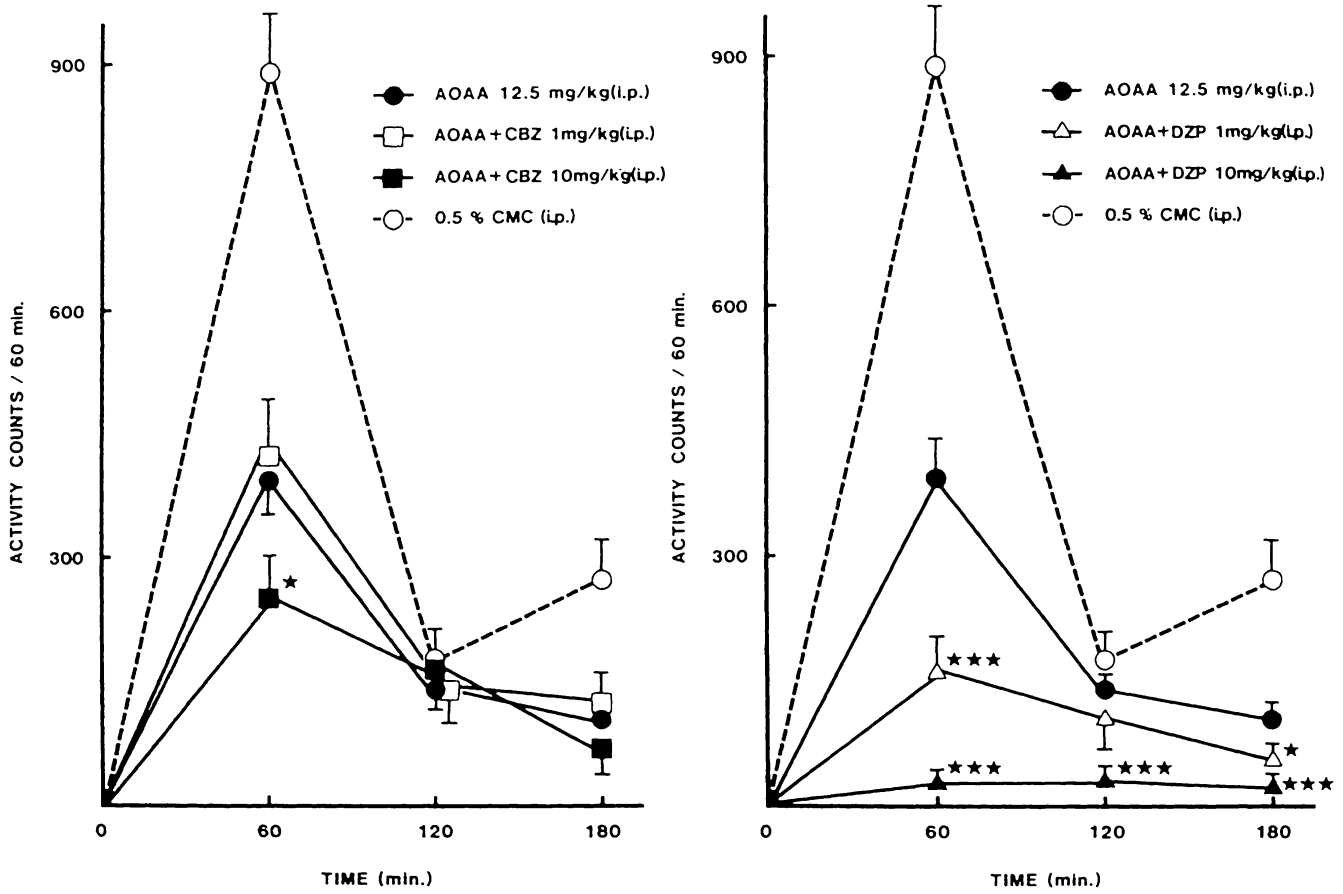

Fig. 7 Effects of CBZ and DZP on changes in locomotor activity produced by AOAA. AOAA was given in a dose of 12.5 mg/kg 3 h before CBZ or DZP. All drugs were injected i.p. For details see legend for Fig. 2. The asterisks indicate values that are significantly different from the corresponding results for AOAA administration (* $p < 0.05$, *** $p < 0.001$).

The effects of clobazam and diazepam on 5-HTP-produced hypomotility

Figure 5 shows that rats treated with 5-HTP alone displayed not only a significant decrease in locomotor activity ($p < 0.001$), but also a sedation of native behavior. 5-HTP-induced sedation was significantly antagonized by clobazam and diazepam. Hypomotility produced by 5-HTP was significantly reversed by clobazam (10 mg/kg) during the first 60-min session ($p < 0.05$). Clobazam (1 mg/kg) was even more potent ($p < 0.001$) at 60 to 120 min after treatment, and forepaw padding was observed. While diazepam (1 mg/kg) reversed hypomotility during the first 60-min session ($p < 0.01$), the 10 mg/kg dose was ineffective ($p < 0.05$).

The effects of clobazam and diazepam on p-CPA-produced locomotor activity

Rats pretreated with p-CPA alone tended to behavioral arousal, including increased locomotion. The locomotor activity produced by p-CPA was inhibited by clobazam (1 and 10 mg/kg) ($p < 0.01$ and $p < 0.05$, respectively), and diazepam (1 and 10 mg/kg) ($p < 0.001$ and $p < 0.01$, respectively) (Fig. 6). The arousal behavior was also completely abolished.

Table 2 Effects of picrotoxin on the locomotor activity of AOAA-treated rats.

Treatment (mg/kg)	No. of rats	Locomotor activity (counts/60 min)
(a) Control	24	892±80
(b) Picrotoxin (0.25 mg/kg)	10	626±77
(c) AOAA (12.5 mg/kg)	10	396±40
(d) AOAA + Picrotoxin	10	545±53

AOAA was given intraperitoneally 3 h before picrotoxin s.c. and the recordings were started immediately after the latter injection. For details see legend for Table 1. Significance: (a) vs (b): $p > 0.05$, (c) vs (d): $p < 0.05$, (a) vs (c): $p < 0.001$.

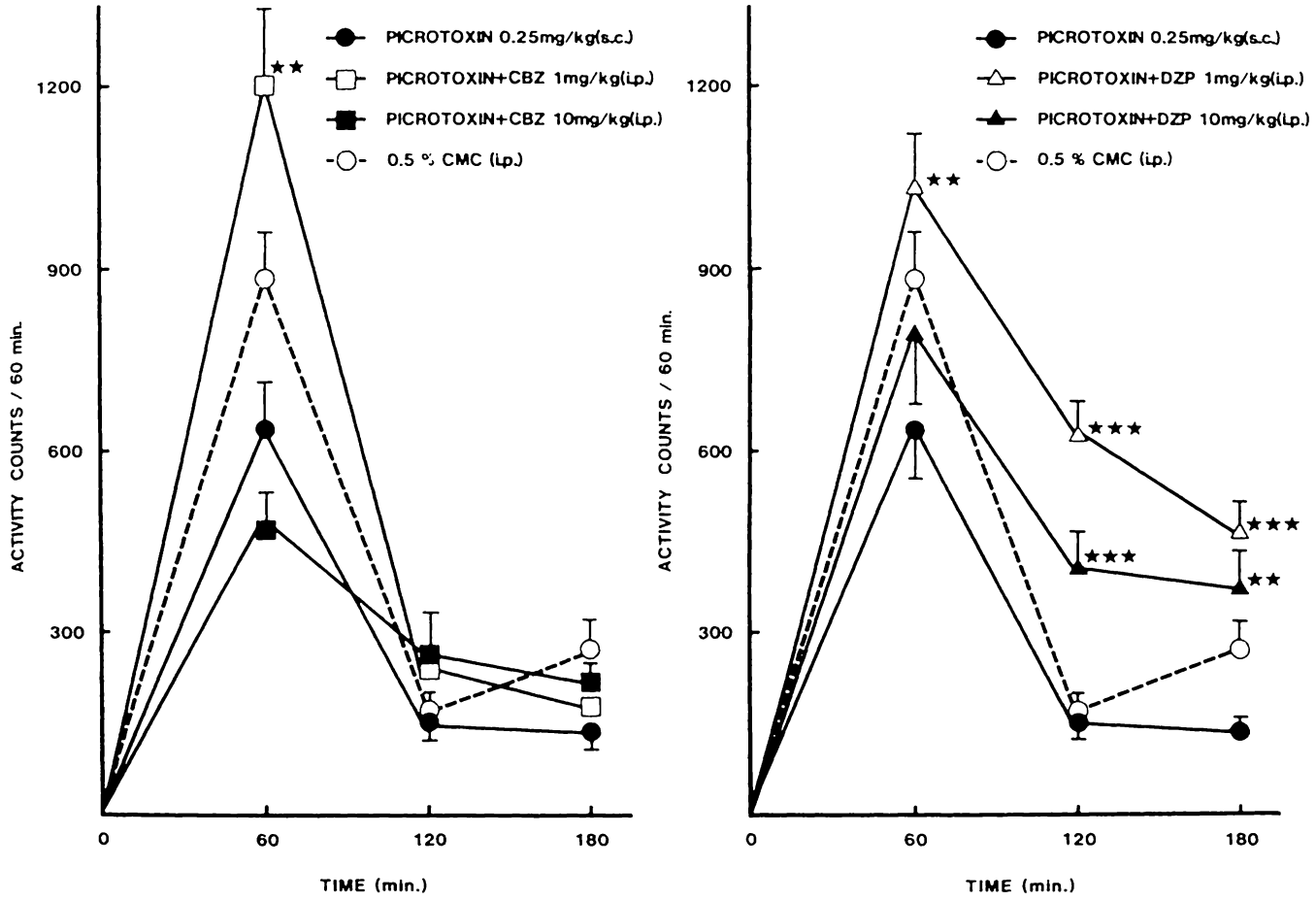

Fig. 8 Effects of CBZ and DZP on changes in locomotor activity produced by picrotoxin. Picrotoxin was given in a dose of 0.25 mg/kg s.c. 10 min before CBZ or DZP injection i.p. For details see legend for Fig. 2. The asterisks indicate values that are significantly different from the corresponding results for picrotoxin administration (** $p < 0.01$, *** $p < 0.001$).

The effects of clobazam and diazepam on AOAA-produced hypomotility

Rats pretreated with the GABA agonist AOAA alone showed markedly significant suppression of locomotor activity ($p < 0.001$) as compared with the 0.5% CMC control and appeared to be behaviorally inactive. The hypomotility produced by AOAA was not affected by clobazam (1 mg/kg), but was enhanced by clobazam (10 mg/kg) during the first 60-min session ($p < 0.05$). However, hypomotility was markedly enhanced between 60 and 180 min by diazepam (1 and 10 mg/kg) in a dose-dependent manner (Fig. 7).

The effects of clobazam and diazepam on picrotoxin-produced hypomotility

Rats pretreated with the GABA antagonist picrotoxin alone exhibited slightly decreased locomotion, but the degree was not statistically different from the 0.5% CMC control ($p > 0.05$). On the other hand, picrotoxin significantly reversed AOAA-produced depression ($p < 0.05$) (Table 2). As Fig. 8 shows, picrotoxin-induced suppression was significantly reversed by clobazam (1 mg/kg) during the first 60-min session ($p < 0.01$), but clobazam (10 mg/kg) was ineffective, while diazepam (1 and 10 mg/kg) markedly and significantly reversed the intensity and duration of the effect of picrotoxin ($p < 0.01$ to $p < 0.001$) between 60 and 180 min.

Discussion

An acute administration of clobazam has been reported to result in a dose-dependent decrease in locomotor activity in mice [Barzaghi et al. 1973]. Our study similarly indicated that single injections of clobazam and diazepam can produce locomotor depression, but clobazam was much less effective than diazepam. On the other hand, Rastogi et al. [1977] found that clobazam produced no significant behavioral change in the rat. The difference between our data and theirs may be due to several kinds of factors, including the apparatus used, route of

administration, and other experimental conditions. Moreover, there is the possibility that the CNS mechanisms involved in locomotor depression by clobazam differ from those with diazepam. Therefore, the present study was carried out to differentiate the involvement of central monoaminergic systems and GABAergic systems in the effects of clobazam and diazepam on motor performance in the rat.

This study indicated that clobazam differs from diazepam in catecholaminergic activity. MAPT, known to exert central stimulant effects, may act via catecholamine (CA) release from nerve terminals [Dominic and Moore 1969, Thornburg and Moore 1973]. In our study diazepam specifically suppressed increased locomotor activity induced by MAPT, but such a response was not influenced by clobazam. In addition, locomotor stimulation occurred, as in other studies [Przegalinski and Kleinrok 1972, Shibuya and Takahashi 1977], by administration of L-dopa plus benserazide, and was significantly attenuated by diazepam but not affected by clobazam. Moreover, slight hypomotility induced by α-MT, an inhibitor of tyrosine hydroxylase [Spector et al. 1965, Moore and Dominic 1971, Widerlöv and Lewander 1978], was markedly augmented by both clobazam and diazepam.

Our data suggest that acute administration of clobazam or diazepam may disturb CA function, and, furthermore, that diazepam has a more pronounced effect than clobazam on catecholaminergic neurons where it probably acts antagonistically.

We secondly investigated how these two benzodiazepines act on 5-HT neurons. The administration of 5-HTP, a precursor of 5-HT, known to increase 5-HT levels in the central nervous system when given alone, has been shown to induce sedation and decrease locomotor activity [Udenfriend et al. 1957, Modigh 1972, Everett 1974], whereas p-CPA, an inhibitor of serotonin synthesis, can cause insomnia, altered patterns of motor activity, and catecholaminergic arousal [Koe and Weisman 1966, Borbély et al. 1973, Mabry and Campbell 1973]. Our data is consistent with these results. An increase in 5-HT activity may therefore be associated with locomotor inhibition, whereas a reduction in 5-HT activity is expected to produce the opposite effect.

Clobazam markedly and significantly reversed the intensity and duration of 5-HTP-induced hypomotility when administered concomitantly, whereas diazepam produced similar yet less pronounced effects. In rats pretreated with p-CPA, locomotor activity was inhibited by both clobazam and diazepam. Furthermore, other investigators have reported that reduction of 5-HT turnover was involved in the anxiolytic activity of benzodiazepines, and that the decrease in CA turnover may be associated with behavioral sedation [Rastogi et al. 1977, Wise et al. 1972]. Our present results support the hypothesis that benzodiazepines elicit their central effects by lowering the turnover and synthesis of brain 5-HT, and suggest that clobazam appears to exert a more remarkable effect than diazepam on serotoninergic transmission where it presumably manifests an antagonistic action.

Thirdly, we attempted to clarify the relationship between the depressive action of clobazam or diazepam and their effects on the GABAergic system. Some investigators have reported that diazepam acts, at least in part, by a specific increase in GABA-mediated inhibition in the central nervous system [Costa et al. 1975, Curtis and Johnson 1974, Geller et al. 1978], and that benzodiazepines could selectively potentiate the effects of both exogenous and synaptically released GABA [Macdonald and Barker 1978, Schacht and Bäcker 1979]. Indeed, there is a substantial amount of evidence suggesting that GABA might be associated with the muscle relaxant, anticonvulsant, and ataxic effects of benzodiazepines [Haefely et al. 1975, Costa et al. 1976]. In the present investigation, when diazepam was combined with AOAA, an inhibitor of GABA-transaminase [Wallach 1961], the intensification of their depressant effects was markedly and significantly synergistic, while clobazam produced similar but less intense effects. Diazepam markedly and significantly reversed the intensity and duration of the effect of picrotoxin, whereas clobazam produced similar but milder effects. Picrotoxin also altered AOAA-induced depression. Our results suggest that diazepam exerts a more marked effect than clobazam on the GABAergic system, where it presumably manifests a synergistic effect.

Taken together, these results strongly suggest that the behavioral depressant effect of benzodiazepines in the rat are possibly mediated, at least partially, by reducing catecholaminergic and serotoninergic activity, and/or by increasing GABAergic activity in the central nervous system. Clobazam is more effective than diazepam in reducing serotoninergic activity, but it is much less effective than diazepam in reducing catecholaminergic activity and in increasing GABAergic activity. However, whether the behavioral suppression of benzodiazepines on cerebral monoaminergic systems is primary to or secondary to their effect on the GABAergic system is less clear. We concluded that differences in the pharmacologic activity of these two drugs may be due to distinct influences on monoaminergic and GABAergic system.

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