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Reversal by alpha-2 agonists of diazepam withdrawal hyperactivity in rats

Joseph Kunchandy and S.K. Kulkarni
Pharmacology Division, Department of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India

Abstract. Rats were made diazepam dependent by chronic treatment with daily injections of the drug, 20 mg/kg, IP, for 3 weeks. On abrupt termination of the drug, the animals showed withdrawal hyperactivity which was indicated by increased horizontal locomotion and vertical activity, and diarrhoea. The peak effect was seen 3 days after the withdrawal of diazepam. Effects of various alpha$_2$ agonists, clonidine, guanfacine and B-HT 920, were studied on the diazepam withdrawal phenomena. Clonidine (100 μg/kg, IP) given twice a day at an interval of 12 h prevented both withdrawal-induced hyperactivity and diarrhoea. On the contrary, equimolar doses of guanfacine and B-HT 920 failed to reverse withdrawal-induced hyperactivity but attenuated the effect of diarrhoea. However, higher doses (500 μg/kg, IP) of guanfacine and B-HT 920 given twice a day at 12-h intervals were found to be effective. Pretreatment with yohimbine (1.5 mg/kg, IP) reversed the protective effect of clonidine, indicating the involvement of alpha$_2$ receptors in the action of clonidine.

Key words: Chronic diazepam – Withdrawal hyperactivity – Diarrhoea – Clonidine – Guanfacine – B-HT 920 – Rat

The benzodiazepine class of drugs are extensively used to treat anxiety, convulsions, sleep disorders, muscle spasm and alcohol withdrawal. They are believed to be the safest of the minor tranquillisers, although they do have potential for physiological and psychological dependence (Petursson and Lader 1981; Tyrer et al. 1983). They are not only used concurrently with major tranquillisers and antidepressants to alleviate anxiety symptoms associated with schizophrenia and depression but are also used chronically in the continued presence of anxiety and insomnia. Recent studies indicated that benzodiazepine treatment shows evidence of dependence (Hallstrom and Lader 1981; Petursson and Lader 1981; Tyrer et al. 1983) and withdrawal reactions on abrupt termination of chronic diazepam treatment in both humans and animals (Murphy et al. 1984; Martin et al. 1982). It was also observed that treatment with Ro 15-1788, a benzodiazepine receptor antagonist, precipitated withdrawal symptoms in baboons (Lamb and Griffiths 1985) and in rats (Cumin et al. 1982; Lukas and Griffiths 1982), indicating a physiological role of benzodiazepine receptors in the development of dependence.

Offprint requests to: S.K. Kulkarni

Recently, clonidine has been advocated in opiate (Gold et al. 1978) and alcohol (Wartenburg 1983) withdrawal syndrome in human as well as in animals. In the present study, we selected two more alpha$_2$ agonists which are reported to be more specific in alpha$_2$ binding than clonidine (Jarrott et al. 1982; Mottram 1983), and studied their effect on diazepam withdrawal symptoms in rats chronically treated with diazepam.

Materials and methods

Adult male Wistar rats weighing 200–250 g (PGI strain) and bred in our animal colony were employed for the present study. They were maintained on rat chow (Hindustan Lever Products) and had free access to water.

Chronic diazepam treatment schedule. Daily injections of diazepam, 20 mg/kg, was given for 3 weeks. Control animals received a proportional quantity of 1% carboxymethylcellulose as vehicle. The injections were made daily between 8 a.m. and 9 a.m. Animals were weighed at 10-day intervals.

Drug treatment schedule. On completion of chronic treatment with diazepam for 3 weeks, the rats were divided into different groups for drug treatment and each group consisted of a minimum of five animals.

The diazepam-withdrawn animals in different groups received two doses of clonidine (50 μg/kg and 100 μg/kg), guanfacine (106 μg/kg and 500 μg/kg) and B-HT 920 (106 μg/kg and 500 μg/kg). The 106 μg/kg dose of guanfacine and B-HT 920 was equivalent to 100 μg/kg clonidine on a molar basis. Each rat received two injections at 12-h intervals, the first injection being made at 8.00 a.m. No drug treatment was given to animals in the control group after diazepam withdrawal.

In another set of experiments, chronically diazepam-treated rats were pretreated with yohimbine (1.5 mg/kg) 30 min prior to each clonidine (100 μg/kg) injection. The effect of yohimbine (1.5 mg/kg) alone was also studied in chronically diazepam-treated rats.

A group of rats receiving only the diazepam injection vehicle (1% carboxymethylcellulose) for 3 weeks served as untreated control. The effects of clonidine, guanfacine and B-HT 920 alone were also studied in non-diazepam-treated animals in order to assess any effect of these agents on normal behaviour.

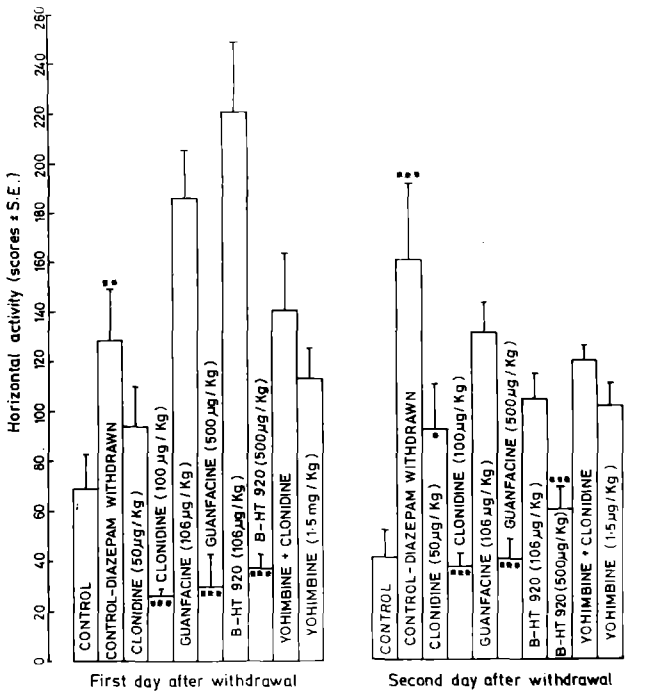

Fig. 1. Effect of clonidine, guanfacine, B-HT 920 and modification of clonidine (100 µg/kg) effect by yohimbine (1.5 mg/kg) on horizontal activity of rats on the 1st and 2nd day of diazepam withdrawal measured in a photoactometer for a period of 10 min (* $P<0.05$; ** $P<0.025$; *** $P<0.005$). Each *bar* represents mean ± SE of a minimum of five observations

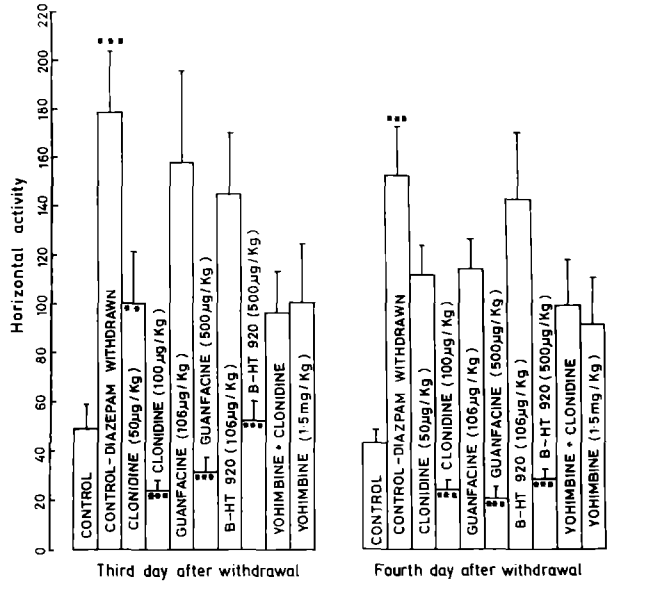

Fig. 2. Effect of clonidine, guanfacine, B-HT 920 and modification of clonidine (100 µg/kg) effect by yohimbine (1.5 mg/kg) on horizontal activity of rats on the 3rd and 4th day of diazepam withdrawal measured in a photoactometer for a period of 10 min (** $P<0.025$; *** $P<0.005$). Each *bar* represents mean ± SE of aminimum of five observations

Measurement of withdrawal symptoms. The withdrawal hyperactivity symptoms were measured using a photoactometer (Techno) for measuring horizontal activity (Dews 1953) and on activity wheel (Techno/modified) for measuring vertical activity of the animals. The activity wheel consisted of a rotating cage wheel with a diameter of 28 cm,

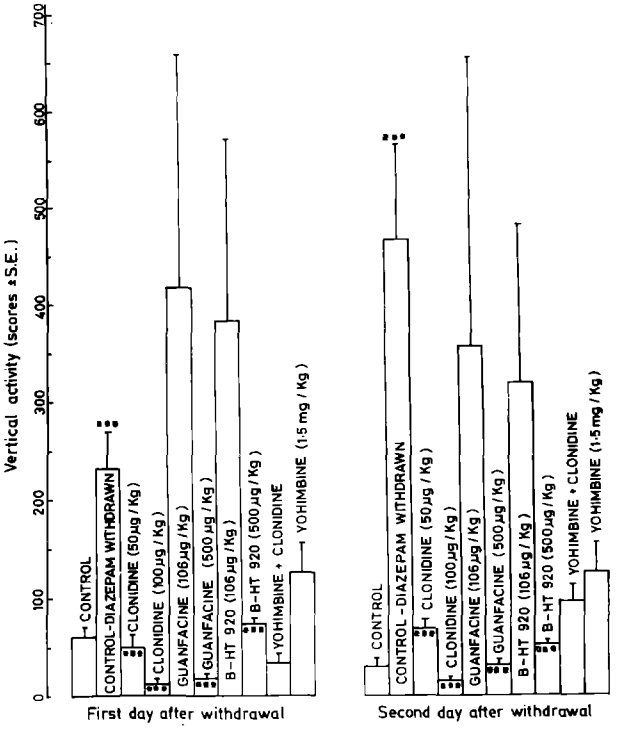

Fig. 3. Effect of clonidine, guanfacine B-HT 920 and modification of clonidine (100 µg/kg) effect by yohimbine (1.5 mg/kg) on vertical activity of rats on the 1st and 2nd day of diazepam withdrawal measured in an activity wheel for a period of 10 min (*** $P<0.005$). Each bar represents mean ± SE of a minimum of five observations

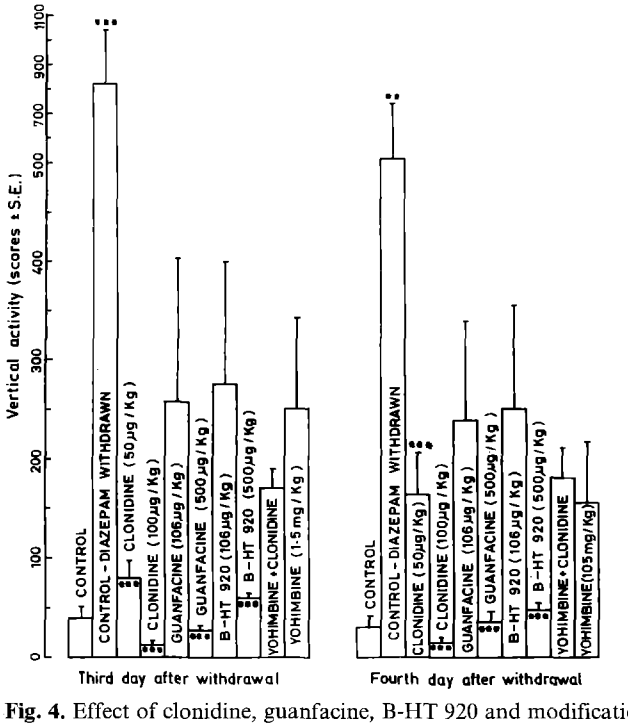

Fig. 4. Effect of clonidine, guanfacine, B-HT 920 and modification of clonidine (100 µg/kg) effect by yohimbine (1.5 mg/kg) on vertical activity of rats on the 3rd and 4th day of diazepam withdrawal measured in an activity wheel for a period of 10 min. (** $P<0.025$; *** $P<0.005$). Each bar represents mean ± SE of a minimum of five observations

Table 1. Horizontal activity (H) and vertical activity (V) of rats as measured in a photoactometer and activity wheel, respectively, for a period of 10 min. The effect of clonidine, guanfacine and B-HT 920 were seen in control non-diazepam-treated rats. The drugs were given twice a day at 12-h intervals

No.	Treatment (dose)	*n*	1st day		2nd day		3rd day		4th day	
			H (mean ± SE)	V (mean ± SE)	H (mean ± SE)	V (mean ± SE)	H (mean ± SE)	V (mean ± SE)	H (mean ± SE)	V (mean ± SE)
1.	Control	6	40.2 ± 6.88	11.1 ± 3.2	25.0 ± 1.4	16.2 ± 2.4	54.8 ± 16.2	53.0 ± 14.2	21.0 ± 4.6	12.0 ± 0.91
2.	Clonidine (100 µg/kg)	5	31.6 ± 5.8	8.9 ± 3.88	23.9 ± 2.1	13.1 ± 4.2	36.7 ± 3.8	30.6 ± 4.1	38.4 ± 6.3	19.8 ± 3.4
3.	Guanfacine (500 µg/kg)	5	36.8 ± 4.2	18.8 ± 3.3	53 ± 11.8	20.6 ± 7.1	98.6 ± 7.3	36.4 ± 2.8	41.8 ± 11.2	31.3 ± 4.8
4.	B-HT 920 (500 µg/kg)	5	43.8 ± 8.9	20.1 ± 4.8	56.1 ± 6.1	43.8 ± 5.1	78.3 ± 4.3	68.8 ± 4.9	86.2 ± 11.3	59.0 ± 8.9

The readings were taken 2.5 h after drug administration

and 14 cm wide. Animals were placed individually inside the cage wheel and as the animal climbed the curvature (vertical activity), the wheel moved down. The movements/rotation of the cage wheel were counted by a photoelectric device. All the animals in various treatment groups were made accustomed to both pieces of apparatus by taking four trial readings in 4 days before the actual measurement.

In the drug-treated groups, the activity was recorded daily 2.5 h after the first dose of the drug. A cut-off time of 10 min was kept constant for both the types of hyperactivity studies. The withdrawal hyperactivity was studied for 4 days after the termination of chronic diazepam treatment.

Drugs: Diazepam (Ranbaxy, New Delhi) was uniformly dispersed in 1% carboxymethylcellulose, clonidine (Boehringer, Ingelheim, FRG), guanfacine (Sandoz, Switzerland), B-HT 920 (Boehringer, Ingelheim, FRG) and yohimbine (E. Merck) were dissolved in distilled water. Injection volume 5 ml/kg was kept constant. All drugs were administered IP.

Statistical analysis. Statistical analysis was done using Student's *t* test.

Results

Chronic diazepam treatment. Daily injections of diazepam 20 mg/kg resulted in tolerance to the sedative effects of the drug, which were seen in the beginning of the treatment as the animals showed reduced locomotion in their home cages. Chronic treatment resulted in weight gain due to hyperphagia.

Diazepam withdrawal symptoms. On abrupt termination of daily injections of diazepam for 3 weeks, the animals manifested hyperlocomotion and hyperactivity sometimes considered as severe anxiety. The peak withdrawal hyperlocomotion, represented as horizontal activity (178.3 ± 25.3) and vertical activity (816.1 ± 221.8), was seen on the 3rd day of diazepam withdrawal (Figs. 2, 4). Besides the increase in hyperactivity, diarrhoea was observed in all the drug-withdrawn animals from the 2nd day of withdrawal. Animals were hyperirritable, showing vocalization (squeaking) on touching.

Effect of clonidine, guanfacine and B-HT 920 on diazepam withdrawal hyperactivity. Clonidine treatment (50 µg/kg and 100 µg/kg, IP) significantly abolished diazepam withdrawal-induced hyperlocomotion (Figs. 1–4). A reduction in both types of activities was recorded. Clonidine treatment also prevented the withdrawal -induced diarrhoea.

Guanfacine and B-HT 920 in lower doses (106 µg/kg, IP) were ineffective in reversing withdrawal hyperactivity, although a prominent antidiarrhoeal action was observed at this dose level. However, at a higher dose level (500 µg/kg, IP) both the drugs showed antiwithdrawal action as they reduced the increased horizontal and vertical activity seen on diazepam withdrawal in rats (Figs. 1–4).

Effect of pretreatment with yohimbine on clonidine-induced protective effects. Pretreatment with yohimbine (1.5 mg/kg) 30 min prior to each clonidine (100 µg/kg) injection significantly reversed its antiwithdrawal effects (Fig. 1–4). However, yohimbine by itself showed a decrease in both variables. The antidiarrhoeal effect of clonidine was also reversed by yohimbine pretreatment.

Effect of clonidine, guanfacine and B-HT 920 on non-diazepam treated animals. Control animals received clonidine, guanfacine and B-HT 920 twice a day at 12-h intervals. The activity was recorded 2.5-h after the first dose of the drug. No significant decreases in any variables were observed when compared with control non-diazepam-treated rats but an increase in the parameters was observed at certain intervals (Table 1).

Discussion

On abrupt termination of chronic diazepam treatment, withdrawal symptoms were noted in rats which were mainly manifested as increased horizontal activity, vertical activity and diarrhoea. Increased anxiety has been reported with diazepam withdrawal in patients treated with 5 mg/kg diazepam for 6 weeks (Murphy et al. 1984). Since it is difficult to quantify the behavioral responses such as anxiety, in our study, we selected two variables which can be easily assessed using automated equipment. Withdrawal hyperactivity was measured as an increase in horizontal or vertical

activity. Diarrhoea was seen as a prominent withdrawal symptom.

The peak withdrawal symptoms were seen on the 3rd day of diazepam termination. This could well be correlated with the long half life (20–70 h) and high protein binding (96–98%) capacity of the drug (Williams et al. 1985).

Clonidine (50 μg/kg and 100 μg/kg) given twice a day at 12-h intervals significantly reduced hyperactivity, as observed in the photoactometer and activity wheel tests following diazepam withdrawal. Treatment with guanfacine (106 μg/kg, twice daily) and B-HT 920 (106 μg/kg twice daily) failed to show any such protective effect, although antidiarrhoeal effect was seen. However, guanfacine and B-HT 920 were effective at the higher dose level (500 μg/kg, twice daily).

Clonidine is reported to be effective in alcohol (Wartenburg 1983) and opiate (Gold et al. 1978) withdrawal symptoms. Guanfacine and B-HT 920 are shown to be more specific in alpha$_2$ receptor binding (Scholtysik 1980; Kobinger and Pichler 1980), but guanfacine and B-HT 920 were found to be less effective than clonidine in the present study. This discrepancy could be due to a possible difference in their sites of action as well as in the pharmacodynamic properties of these agonists. Guanfacine is reported to have less sedative action as compared to clonidine (Scholtysik et al. 1975). The drugs also differ in their effects on dopamine turnover in rat brain (Scholtysik et al. 1975; Saameli et al. 1975). Topical application of clonidine to the exposed ventral surface of the medulla oblongata in cats induced a marked fall in the mean arterial blood pressure and heart rate (Bousquet and Guertzenstein 1973; Scholtysik et al. 1975), while guanfacine was ineffective in this test model (Scholtysik et al. 1975), suggesting that the sites of action within the CNS may be different for the two drugs.

However, the alpha$_2$ adrenoceptor involvement in the effect of clonidine in diazepam withdrawal is supported by reversal of its effect by yohimbine pretreatment.

The sedative effect of clonidine, which is not seen with guanfacine and B-HT 920, does not seems to play a part in the protective effect. Moreover, the withdrawal symptoms were measured 2.5-h after drug administration. There was no significant decrease in the parameters in control animals which received clonidine, guanfacine or B-HT 920, but an increase in the parameters was seen at certain intervals (Table 1).

Withdrawal-induced diarrhoea was consistantly abolished by all the three alpha$_2$ agonists studied. The stimulation of intestinal alpha$_2$ receptors is reported to reduce the motility and secretion of the gastrointestinal tract (Dijoseph et al. 1984).

Long-term daily injections of diazepam are reported to induce a selective subsensitivity to microiontophoretically applied GABA in serotonergic dorsal raphe neuron in the rat, which may be reversed by Ro 15-1788 (Gallager et al. 1984; Gonsalves and Gallager 1985). This subsensitivity persisted for 96-h after the final dose of diazepam, even though the agonist and its active metabolites were no longer detectable in brain tissue. Martin et al. (1982) reported that the course of the diazepam abstinence syndrome in rats roughly parallels the time course of reversal of the subsensitivity to GABA. Thus it is speculated that the decreased responsiveness to GABA in the absence of diazepam may contribute directly or indirectly to the behavioral and neurological signs of hyperexcitability seen after abrupt discontinuation of high doses of benzodiazepines (Penvick et al. 1978; Winokur et al. 1980; Martin et al. 1982; Ryan and Boisse 1983; McNicholas et al. 1983).

Modulation of the GABAergic system by alpha$_2$ adrenoceptors is not known. However, the involvement of a non-GABAergic mechanism in benzodiazepine withdrawal is possible (Fariello and Ticku 1983); if only secondary to altered GABA function. Since clonidine, guanfacine and B-HT 920 are shown to be effective in diazepam withdrawal hyperactivity, this suggests increased noradrenergic activity in diazepam withdrawal. However, this could be secondary to altered GABA function, as GABA has an inhibitory effect on the firing of noradrenergic neurons. The unpublished biochemical data from our laboratory showed a significant increase in brain monoamine levels, particularly NE and 5-HT, on diazepam withdrawal when compared with chronic diazepam-treated brain tissues of rats, further supporting the above observations.

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