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Role of central histaminergic system in lorazepam withdrawal syndrome in rats

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Abstract

Effects of histaminergic agonists and antagonists were investigated on withdrawal signs in lorazepam-dependent rats. Physical dependence was developed by giving lorazepam admixed with the food in the following dose schedule (in mg/kg given daily × days): 10 × 4, 20 × 4, 40 × 4, 80 × 4, and 120 × 7. The parameters observed during the periods of administration of lorazepam and after its withdrawal were spontaneous locomotor activity (SLA), reaction time to pain, foot shock aggression (FSA), and audiogenic seizures. During the withdrawal period, the rats were divided into groups of 10 each. Control-withdrawal group did not receive any drug. The drugs (in mg/kg administered intramuscularly) — L-histidine (50), histamine-*N*-methyl (2), promethazine (10), pheniramine (10), astemizole (10), and thioperamide (1) — were given separately in other groups daily during the withdrawal period. The withdrawal signs in control group were hyperkinesia, hyperaggression, and audiogenic seizures. L-Histidine, precursor of histamine, and thioperamide, antagonist of H3 receptor, potentiated hyperkinesia, hyperaggression, and audiogenic seizures. Histamine-*N*-methyl, agonist of H3 receptor, and H1 receptor antagonists, promethazine and pheniramine, blocked all the withdrawal signs. Astemizole, a peripheral antagonist of H1 receptor, could not affect any withdrawal sign. It may be concluded that histamine H1 receptors are facilitatory and H3 receptors are inhibitory for benzodiazepine (BZD) withdrawal syndrome.

Keywords: Benzodiazepine; Physical dependence; Withdrawal syndrome; Histamine; H1 receptor; H3 receptor

1. Introduction

The entity of histamine as central neurotransmitter is well established. The presence of histamine neurons and it receptors subtypes (H1, H2, and H3) in the brain has been demonstrated (Schwartz et al., 1995). Certain behavioral responses are influenced by central histaminergic receptors (Nath et al., 1982, 1988). However, the role of histaminergic system in drug dependence has not been elucidated. In the present endeavor, histaminergic system was investigated for its possible role in benzodiazepines (BZD) dependence. The physical dependence on BZD, the most frequently prescribed group of antianxiety drugs, is now a recognized fact in man and in different species of animals — monkey, dog, and rodent (Howe, 1980; Tyrer and Seivewright, 1984; Wood et al., 1995). Most of the studies on BZD dependence were mainly concerned with the development of physical dependence characterized by withdrawal syndrome. BZD are known to interact with other neurotransmitters through GABA–BZD Cl^- ionophore complex (Hobbs et al., 1996) but central neurotransmitters do not receive much attention in BZD dependence. We, indeed, reported the involvement of dopaminergic system in BZD dependence in rats (Nath et al., 2000). In our earlier studies, diphenhydramine, an antagonist of histamine H1 receptor, was found to affect the dependence induced by methaqualone and lorazepam in rats (Nath et al., 1994, 1997). Comparative evaluation of dependence liability of BZD with different half-life showed that lorazepam was the most suitable one to induce physical dependence in rats on the basis of severity and consistency of withdrawal signs (Gupta et al., 1993). Therefore, lorazepam has been taken as the representative drug among BZD in the present study to investigate the effect of histaminergic drugs on the withdrawal syndrome of BZD in the rats.

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2. Method

2.1. Drugs

The drugs used were lorazepam (Cipla, India), pheniramine (Torrent, India), promethazine (Torrent), astemizole (Torrent), L-histidine (RBI, USA), histamine-*N*-methyl (RBI), and thioperamide (RBI). Their doses (in mg/kg, given daily) were histamine precursor L-histidine (50), H3 receptor agonist histamine-*N*-methyl (2), H1 receptor antagonists pheniramine (10), promethazine (10), and astemizole (10), and H3 receptor antagonist thioperamide (1). Each drug was dissolved in normal saline and administered in separate group by intramuscular route.

2.2. Animal

The study was conducted on male Wistar rats (weighing 150–180 g). Each rat was fed daily with a special diet comprising gram flour (9 g), milk powder (0.9 g), and glucose (0.1 g) mixed with 20 ml of water in deep containers to avoid spillage. It has been our observation that 20 ml of this diet is more than the daily requirement. Water was available ad libitum. The animals were housed in a room with a light–dark cycle of 12 h.

2.3. Development of physical dependence on lorazepam

The present study was conducted in two sets — one for agonists and another for antagonists of histaminergic system. In each set lorazepam was administered daily for 23 days after uniformly mixing with the food, as described by Yanura et al. (1975). The dose schedule of lorazepam used was (in mg/kg daily × days) 10×4, 20×4, 40×4, 80×4, and 120×7 (Gupta et al., 1996). The average amounts of lorazepam consumed by the rats of both the sets, calculated on the basis of daily food intake were (in mg/kg daily × days) 8.7×4, 17.8×4, 34.1×4, 70.2×4, and 98.6×7. The withdrawal syndrome was observed after cessation of lorazepam administration. In each set during the withdrawal period, the rats were divided into groups of 10 each. One group did not receive any drug and served as control-withdrawal, while other groups received histaminergic drugs in their pharmacologically effective doses daily during the withdrawal period. The drugs and their dose (in mg/kg given daily) were histamine precursor L-histidine (50), H3 receptor agonist histamine-*N*-methyl (2), H1 receptor antagonists pheniramine (10), promethazine (10), and astemizole (10), and H3 receptor antagonist thioperamide (1). Each drug was administered in separate group by intramuscular route 30 min prior to the start of experiments.

One group of rats ($n = 10$) was kept only on the diet (no lorazepam) for 33 days and served as lorazepam nondependent control group for each set.

Following responses were observed prior to the start of lorazepam administration (control-Day 0) and during the periods of administration and withdrawal of lorazepam and in nondependent control groups.

2.4. Spontaneous locomotor activity (SLA)

SLA was recorded by photoactometer (Techno) (Dews, 1958). The activity was counted for 5 min after a period of 2-min acclimatization in each rat.

2.5. Pain response

The tail clip method was employed to study pain response (Chen, 1958). The time interval between the application of clip to tail and first biting of the clip by the rats was taken as reaction time to pain. The rats, which had initial reaction time of more than 10 s, were excluded from the study. The cut off time was 30 s in test groups.

2.6. Foot shock aggression (FSA)

Aggression was induced by electric foot shock (2 mA, five shocks per second) by Aggressometer (Techno) to pairs of rats according to the method of Tedeschi et al. (1959). The paired rats were kept in close proximity to each other. The numbers of fighting bouts in upright posture were counted for a period of 1 min.

2.7. Audiogenic seizures

Audiogenic stimuli were given with the help of electric doorbell (sound volume 50 dB) fixed in a metal chamber for 30 s to elicit the seizure (Essig, 1966).

In all the groups, parameters were observed in the forenoon. The order for observation of these parameters was SLA, pain response, FSA, and audiogenic seizures. The parameters were recorded with an interval of 2 min except between FSA and audiogenic seizures where it was 5 min.

Body weight and food intake was recorded daily in all the groups.

The significance of difference between the groups was determined by ANOVA (two-way analysis of variance) and followed by Dunnett's test for SLA, reaction time to pain, and FSA, and chi-square test with Yates correction for audiogenic seizures.

The research work was approved by a committee of Central Drug Research Institute.

3. Results

3.1. Lorazepam administration period

On the last day (i.e., Day 23) of lorazepam administration, the count for SLA and FSA were 25 ± 3.7 and 2 ± 0.4, respectively, which were significantly lower as compared

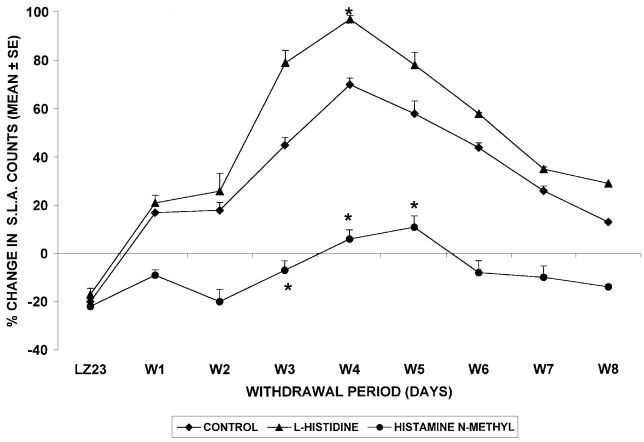

Fig. 1. Effect of histaminergic drugs — histamine precursor L-histidine (50 mg/kg im) and H3 receptor agonist histamine-*N*-methyl (2 mg/kg im) — on SLA during the withdrawal period (W1–W8) of lorazepam. The percent change indicates change in SLA counts from Day 0, i.e., prior to the start of lorazepam administration. LZ 23 = 23rd day (i.e., last day) of lorazepam administration. * Significant difference ($P < .05$) from control values (by Dunnett's test; all data are mean ± S.E.M.).

to control-Day 0 (SLA 81 ± 7.1; FSA 4 ± 0.3) and on 23rd day in nondependent group (SLA 77 ± 10.9; FSA 5 ± 0.5). Pain reaction was not affected significantly. Audiogenic seizures did not appear in any rat. These data are combined of both the sets. There were no significant differences between the responses observed during the lorazepam administration in rats of both the sets. The nondependent controls in both the sets were also not significantly different from each other.

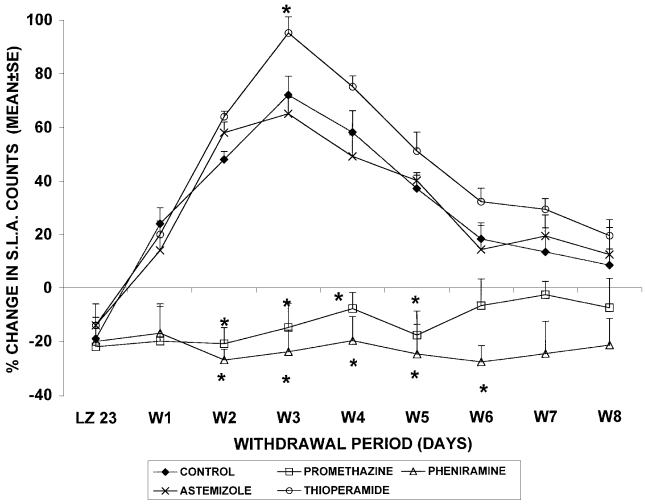

Fig. 2. Effect of histamine antagonists — H1 receptor antagonists pheniramine (10 mg/kg im), promethazine (10 mg/kg im), and astemizole (10 mg/kg im) and H3 receptor antagonist thioperamide (1 mg/kg im) — on SLA during the withdrawal period (W1–W8) of lorazepam. The percent change indicates change in SLA counts from Day 0, i.e., prior to the start of lorazepam administration. LZ 23 = 23rd day (i.e., last day) of lorazepam administration. * Significant difference ($P < .01$) from control values (by Dunnett's test; all data are mean ± S.E.M.).

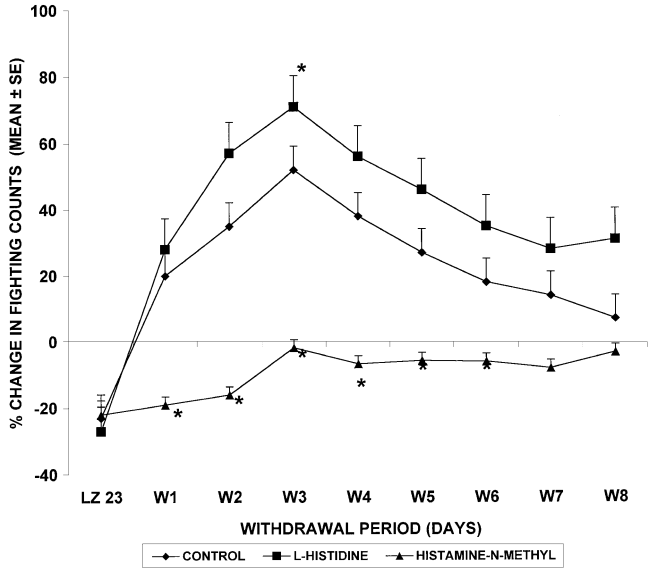

Fig. 3. Effect of histaminergic drugs — histamine precursor L-histidine (50 mg/kg im) and H3 receptor agonist histamine-*N*-methyl (2 mg/kg im) — on FSA during the withdrawal period (W1–W8) of lorazepam. The percent change indicates change in FSA counts from Day 0, i.e., prior to the start of lorazepam administration. LZ 23 = 23rd day (i.e., last day) of lorazepam administration. * Significant difference ($P < .01$) from control values (by Dunnett's test; all data are mean ± S.E.M.).

3.2. Lorazepam withdrawal period

The maximal effects (peak withdrawal) appeared on Days 2–4 and then gradually declined to level of control-Day 0 and nondependent control groups within 8–9 days.

The results on the different parameters are the following.

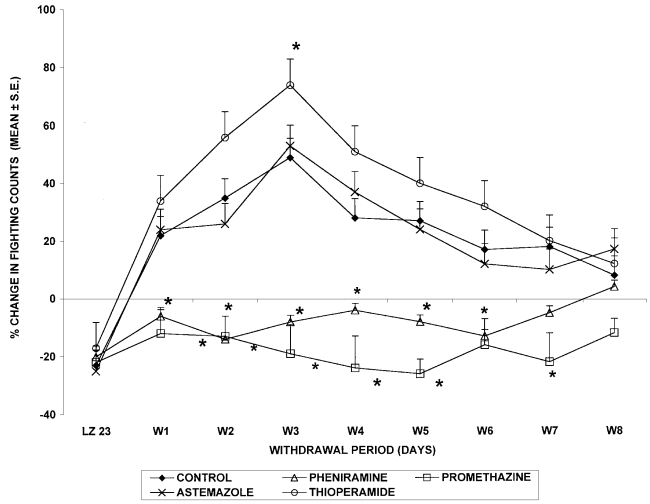

Fig. 4. Effect of H1 receptor antagonists pheniramine (10 mg/kg im), promethazine (10 mg/kg im), and astemizole (10 mg/kg im) and H3 receptor antagonist thioperamide (1 mg/kg im) on FSA during the withdrawal period (W1–W8) of lorazepam. The percent change indicates change in FSA counts from Day 0, i.e., prior to the start of lorazepam administration. LZ 23 = 23rd day (i.e., last day) of lorazepam administration. * Significant difference ($P < .01$) from control values (by Dunnett's test; all data are mean ± S.E.M.).

3.2.1. Spontaneous locomotor activity

There was significant difference in the SLA count between control-withdrawal histaminergic drugs except astemizole (Figs. 1 and 2). As compared to control-Day 0, L-histidine and thioperamide group showed hyperkinesia (95–97% increase in SLA) significantly different from control-withdrawal (69% increase). There was decrease of 20–28% in SLA (hypokinesia) during the withdrawal period of promethazine-, pheniramine-, and histamine-*N*-methyl-treated rats, which was significantly different from control-withdrawal (72% increase).

3.2.2. Foot shock aggression

As compared to control-Day 0, the aggressive response was significantly higher in rats that received L-histidine and thioperamide (71–74% increase) daily during the withdrawal, which was significantly more than control-withdrawal (52% increase) (Figs. 3 and 4). Administration of promethazine, pheniramine, and histamine-*N*-methyl produced 20–26% decrease in FSA (hypoaggression) significantly different from control-withdrawal (49% increase).

3.2.3. Audiogenic seizures

In control-withdrawal group of both the sets, 30% of rats developed audiogenic seizures (Fig. 5). The seizures appeared from the Day 1 of lorazepam withdrawal and persisted till Day 9. The audiogenic seizures were potentiated (50–60% incidence) in the groups that received L-histidine and thioperamide. Promethazine, pheniramine, and histamine-*N*-methyl (10% audiogenic seizures only on second day) afforded 100% protection against audiogenic seizures. Astemizole-treated rats showed the audiogenic seizures similar to control-withdrawal group.

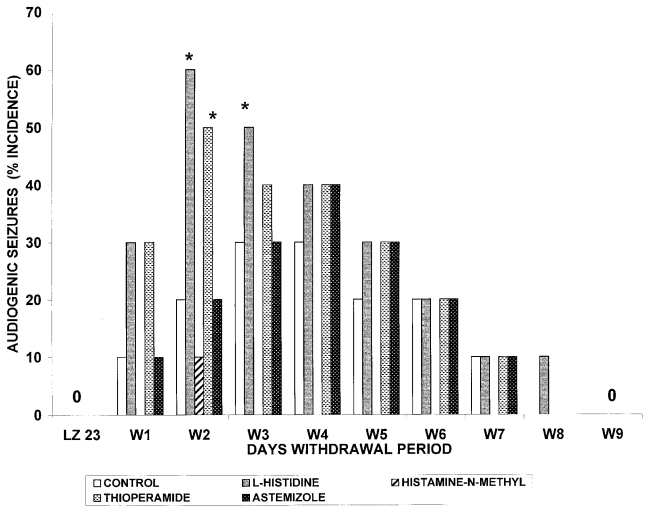

Fig. 5. Effect of histaminergic drugs — histamine precursor L-histidine (50 mg/kg im), H3 receptor agonist histamine-*N*-methyl (2 mg/kg im), peripheral H1 receptor antagonist astemizole (10 mg/kg im), and H3 receptor antagonist thioperamide (1 mg/kg im) — on the incidence of audiogenic seizures during the withdrawal period (W1–W9) of lorazepam. LZ 23 = 23rd day (i.e., last day) of lorazepam administration. The incidence of audiogenic seizures was 0% on LZ 23 day and in H1 receptor antagonists — pheniramine (10 mg/kg im)- and promethazine (10 mg/kg im)-treated rats during the withdrawal period. * Significant difference ($P < .05$) from control values (by chi-square test with Yates correction, $df = 1$, $n = 10$).

There were no significant differences in the reaction time to pain and food intake between the control-Day 0, control-nondependent, and drug-treated groups. The gain in body weight was similar in lorazepam-dependent and nondependent groups.

4. Discussion

That the central histaminergic system exerts significant influence on physical dependence induced by lorazepam has been observed in the present study. Hyperkinesia, hyperaggression, and audiogenic seizures were observed following the cessation of lorazepam administration in the rats that received lorazepam daily in increasing order of doses. These effects are withdrawal signs because these signs are absent during the period of lorazepam administration and subsided by readministration of lorazepam (Nath et al., 1994). Characteristic feature of withdrawal syndrome of psychotropic drugs is neuronal hyperexcitability (O'Brien, 1996) and, as matter of fact, these withdrawal signs, particularly audiogenic seizures, reflect a state of neuronal hyperexcitability (Essig, 1966). It is an accepted view that appearance of withdrawal syndrome confirms the development of physical dependence and severity of withdrawal syndrome indicates the magnitude of physical dependence (O'Brien, 1996). Therefore, involvement of a neurotransmitter in the physical dependence can be ascertained by studying the effects of drugs, acting through that particular neurotransmitter system, on withdrawal signs. Following this strategy, we have determined the role of histamine in lorazepam-induced physical dependence.

Administration of L-histidine (precursor of histamine), that freely crosses the blood–brain barrier, potentiated all the withdrawal signs of lorazepam. This observation indicates the enhancement of histaminergic system aggravates withdrawal signs of lorazepam. Histamine receptors belong to three distinct groups — H1, H2, and H3. The distribution, molecular characterization, and cloning of these histamine receptors are well documented. Histamine H1 and H2 receptors are postsynaptic while H3 is presynaptic (Hill, 1990). The role of histamine receptors in lorazepam-induced physical dependence was delineated by observing the effects of specific antagonists in their known effective doses on withdrawal signs. However, H2 antagonists could not be employed because the currently available H2 antagonists do not cross blood–brain barrier and daily intracerebroventicular administration was not suited to the present experimental design.

H1 receptor antagonists — promethazine and pheniramine — blocked withdrawal signs. The observation was similar to that reported in case of diphenhydramine (Nath

et al., 1997). Astemizole, a peripheral H1 receptor antagonist that cannot freely permeate the blood–brain barrier (Krstenansky and Cluxton, 1987), could not affect significantly any of the withdrawal signs. This indicates the involvement of central, not peripheral, H1 receptors in lorazepam-induced physical dependence. Histamine H1 receptor antagonists are not reported to cause significant behavioral changes except sedation in humans (Wood et al., 1995). Sedation might play a contributory role in reversal of hyperkinesia by H1 antagonists — promethazine and pheniramine — during the withdrawal period. Mepyramine, an H1 antagonist, potentiates FSA in mice (Nath et al., 1982), but in this study, H1 antagonists promethazine and pheniramine inhibited the foot shock hyperaggression that occurred following the withdrawal of lorazepam. It seems that antagonism of hyperaggression of lorazepam withdrawal by H1 antagonists could be due to interference in the mechanism of withdrawal rather than per se effect of H1 antagonists.

Histamine H3 receptor agonist histamine-*N*-methyl inhibited and H3 antagonist thioperamide potentiated the withdrawal signs of lorazepam. H3 receptors are autoreceptors that regulate the release of histamine. Stimulation of the autoreceptor by the agonist decreases the release of neurotransmitter and blockade by the antagonist increases it (Array et al., 1992). Therefore, the inhibition of withdrawal signs by H3 agonist and potentiation of withdrawal signs by H3 antagonists may be attributed to the decrease and increase in the release of histamine, respectively.

In the present study, enhancement in histaminergic activity by precursor L-histidine and release thioperamide (H3 antagonist) potentiated the audiogenic seizures of lorazepam withdrawal and H1 antagonist suppressed the seizures. It has been reported that inhibitory effect of H3 agonists on amygdaloid-kindled seizures is blocked by H1 and H3 antagonists in rats (Kakinoki et al., 1998), but H3 antagonists do not produce pronounced effect against electroshock- and pentylenetetrazol-induced seizures in mice (Fischer and van der Goot, 1998). Histamine inhibits audiogenic seizures in genetically sensitive rats but does not affect ethanol withdrawal-induced audiogenic seizures in rats (Feng and Faingold, 2000). Thus, the facilitatory effect of histamine on audiogenic seizures obtained in this study indicates that histaminergic system in lorazepam withdrawal is involved in a specific manner because it is contrary to the inhibitory influence of histamine observed by other workers on different types of seizures. Finally, it emerges that enhancement in histaminergic activity, by increasing synthesis either through precursor L-histidine or release by H3 (autoreceptor) antagonist thioperamide, potentiates the withdrawal signs whereas central histamine H1 receptor antagonists or release inhibitor H3 agonist histamine-*N*-methyl inhibit them.

In conclusion, the results of the study implicate the histamine system in the physical dependence of lorazepam. It was demonstrated that the histaminergic facilitation, presumably mediated through histamine H1 receptors and H3 receptors, might exert inhibitory influence in the development of withdrawal signs. The study further highlights the significance of central histaminergic system in the regulation of behavioral responses.

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References

Array J-M, Garbarg M, Schwartz J-C. H3-receptor and control of histamine release. In: Schwartz J-C, Hass HL, editors. The histamine receptors. New York: Wiley-Liss, 1992. pp. 145–59.

Chen G. The antiparkinsonian effects of some drugs as determined by Haffner's method of testing analgesia in mice. J Pharmacol Exp Ther 1958;124:73–6.

Dews PB. The measurement of the influence of drugs on locomotor activity of mice. Br J Pharmacol 1958;8:46–8.

Essig CF. Barbiturate withdrawal in white rats. Int J Neuropharmacol 1966; 5:103–7.

Feng HJ, Faingold CL. Modulation of audiogenic seizures by histamine and adenosine receptors in the inferior colliculus. Exp Neurol 2000; 163:264–70.

Fischer W, van der Goot H. Effect of clobenpropit, a centrally acting histamine H3-receptor antagonist, on electroshock and pentylenetetrazol induced seizures in mice. J Neural Transm 1998;105:587–99.

Gupta MB, Nath C, Bhalla DN, Patnaik GK, Dhawan KN. Comparative evaluation of physical dependence of some benzodiazepines in rats. Ann Neurosci 1993;4:13–8.

Gupta MB, Nath C, Patnaik GK, Saxena RC. Effect of calcium channel blockers on withdrawal syndrome of lorazepam in rats. Ind J Med Res 1996;103:310–4.

Hill SJ. Distribution, properties and functional characteristics of three classes of histamine receptor. Pharmacol Rev 1990;40:45–83.

Hobbs WR, Rall TW, Verdoorn TA. Sedatives and hypnotics: ethanol. In: Hardman JG, Limbird LE, Molinoff PB, Rudden RW, Gillman AG, editors. Goodman and Gillman's the pharmacological basis of therapeutics vol. 9. New York: McGraw-Hill, 1996. pp. 361–98.

Howe JG. Lorazepam withdrawal seizures. Br Med J 1980;280:1163–4.

Kakinoki H, Ishizawa K, Fukunaga M, Fujii Y, Kamei C. The effects of histamine H3-receptor antagonists on amygdaloid kindled seizures in rats. Brain Res Bull 1998;46:461–5.

Krstenansky PM, Cluxton RJ. Astemizole: a long-acting non sedating antihistamine. Drug Intell Clin Pharm 1987;21:947–53.

Nath C, Gualti A, Dhawan KN, Gupta GP, Bhargava KP. Evidence for role of central histaminergic system in foot shock induced aggressive behavior. Psychopharmacology 1982;76:228–31.

Nath C, Gulati A, Dhawan KN, Gupta GP. Role of central histaminergic mechanism in behavioural depression (swimming despair) in mice. Life Sci 1988;42:2413–8.

Nath C, Gupta MB, Shanker K, Gupta GP, Srimal RC, Dhawan BN. Effect of diphenhydramine on withdrawal syndrome of some psychoactive drugs. In: Dhawan BN, Srimal RC, Rao RS, Raghubir R, editors. Recent advances in study of neurotransmitter receptor. Lucknow: Central Drug Research Institute, 1994. pp. 310–8.

Nath C, Patnaik GK, Saxena RC, Gupta MB. Evaluation of inhibitory effect of diphenhydramine on benzodiazepine withdrawal. Int J Physiol Pharmacol 1997;41:42–6.

Nath C, Saxena RC, Gupta MB. Effect of dopamine agonists and antagonists on lorazepam withdrawal syndrome in rats. Clin Exp Physiol Pharmacol 2000;27:167–71.

O' Brien CP. Drug addiction and drug abuse. In: Hardman JG, Limbird LE, Molinoff PB, Rudden RW, Gillman AG, editors. Goodman and Gillman's the pharmacological basis of therapeutics. New York: McGraw-Hill, 1996. pp. 581–600.

Schwartz J-C, Array J-M, Garbarg M, Traffort E. Histamine. In: Bloom BE, Kupfer DJ, editors. Psychopharmacology, the fourth generation of progress. New York: Raven Press, 1995. pp. 397–406.

Tedeschi RE, Tedeschi DH, Muchs A, Cook L, Mattis PA, Fellows EJ. Effects of various centrally acting drugs on fighting behaviour of mice. J Pharmacol Exp Ther 1959;125:28–34.

Tyrer PJ, Seivewright N. Identification and management of benzodiazepine dependence. Postgrad Med J 1984;60(Suppl 2):41–6.

Wood JH, Katz JL, Winger G. Abuse and therapeutic use of benzodiazepine and benzodiazepine like drugs. In: Bloom BE, Kupfer DJ, editors. Psychopharmacology, the fourth generation of progress. New York: Raven Press, 1995. pp. 1777–92.

Yanura S, Taqashira E, Suzuki T. Physical dependence on morphine, phenobarbital and diazepam in rats by drug admixed food ingestion. Jpn J Pharmacol 1975;25:453–63.