Pharmacology Biochemistry & Behavior, Vol. 40, pp. 801–805. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

Diltiazem Alters Some Withdrawal Signs in Pentobarbital-Dependent Rats

GERALD J. YUTRZENKA AND MATT REYNEN

Department of Physiology and Pharmacology, University of South Dakota School of Medicine, Vermillion, SD 57069

Received 1 November 1990

YUTRZENKA, G. J. AND M. REYNEN. *Diltiazem alters some withdrawal signs in pentobarbital-dependent rats.* PHARMACOL BIOCHEM BEHAV **40**(4) 801–805, 1991.—Male, Sprague-Dawley rats were made dependent on pentobarbital sodium (PB) by continuous, IP infusion of PB for 13 days. On Day 14, a 72-hour PB-free period began during which body weight, 24-hour water consumption and withdrawal scores were noted. In Study 1, rats were placed into one of four treatment groups at the start of the PB-free period. Groups included saline-infused control rats with twice daily administration of either vehicle or diltiazem and PB-dependent rats treated twice daily with either diltiazem or vehicle. In Study 2, rats were placed into one of the four treatment groups at the start of the 13-day PB-infusion period. In Study 1, PB-dependent rats treated with diltiazem exhibited approximately 10% loss of body weight at both 12 and 48 hours of the PB-free period while PB-dependent rats treated with vehicle exhibited only about a 5% loss of body weight. PB-dependent rats treated with either diltiazem or vehicle both exhibited about a 40% decline in water consumption and were noted to have significant increases in withdrawal scores by the fifth hour of the PB-free period. As compared to the scores of PB-dependent rats treated with vehicle, diltiazem did not significantly alter the withdrawal scores of PB-dependent rats at any time point during the PB-free period. In Study 2, the chronic administration of diltiazem to PB-infused rats produced both a significant decrease in water consumption at 48 and 72 hours and a significant increase in withdrawal scores from 3–48 hours. Mortality, which occurred only during the PB-free period and involved only PB-dependent rats treated with diltiazem, reached 50% by the end of the PB-free period. It appears that the chronic administration of diltiazem for 13 days led to an exacerbation of some of the typical withdrawal symptoms in PB-dependent rats.

Pentobarbital Physical dependence Diltiazem Rats

THE calcium channel blockers have become an important group of therapeutic agents for treatment of hypertension, arrhythmias and angina (13,15). This class of therapeutic agents, represented clinically by such diverse drugs as nitrendipine, verapamil, and diltiazem, direct their action towards the blockade of voltage-dependent calcium channels in the membrane. The inhibition of calcium flux through these channels results in the disruption of such functions as excitation-contraction coupling or the stimulus-secretion coupling events which lead to the release of hormones and neurotransmitters (4, 11, 21, 27).

The calcium channel blockers have been shown to alter the effects of a variety of centrally acting agents. It has been reported that diltiazem, verapamil and nicardipine produced both a dose-dependent analgesic effect as well as an enhanced antinociceptive action of morphine in laboratory rodents (8). Additionally it was noted that these calcium channel blockers reduced the degree of opiate withdrawal symptomology following naloxone administration to opiate-dependent rodents (2, 3, 5). On the other hand, administration of the calcium channel activator, Bay K 8644, significantly increased the degree of withdrawal symptomology observed following naloxone challenge (2). While the opiates and the calcium channel blockers do not appear to act at the same receptor site, it does appear that there is a functional interaction between the sites as morphine administration does appear to lead to an increase in the number of binding sites for calcium channel blockers (2,3).

The calcium channel blockers also have been shown to alter many of the effects of CNS depressant drugs. It has been reported that these agents may reduce the duration of hypnosis induced by ethanol (22,24), barbiturates (20) and benzodiazepines (18). The calcium channel blockers have also been noted to both increase the acute motor incoordination effects of ethanol as well as delay the acquisition of tolerance to some of the effects of ethanol (24). It has been noted that while acute ethanol administration increased levels of ionized calcium and reduced the magnitude of KCl-stimulated calcium release from synaptosomes, the chronic administration of ethanol resulted in a lessening of these effects suggesting the establishment of tolerance to these ethanol-induced effects on ionized calcium (4). Finally, it has been demonstrated that whereas verapamil and nitrendipine potentiated the anesthetic effect of pentobarbital and ethanol, Bay K8644 inhibited the anesthetic action of these CNS depressant drugs (9,10).

The present investigation was designed to assess the effects of the calcium channel blocker, diltiazem, on the withdrawal symptomology associated with the establishment of physical dependence on barbiturates.

METHOD

Male, Sprague-Dawley, rats (Sasco, Omaha, NE) weighing 160–180 g at the start of the study were housed individually. Purina Lab Chow (Ralston Purina Co., St. Louis, MO) and water were available ad lib. Rats were maintained under a 12-hour

on/off light cycle with lights on at 7:30 a.m. All rats were acclimated to the animal care facility for several days prior to use in the study.

Rats were surgically prepared with intraperitoneal cannula (PE90) while under halothane/nitrous oxide anesthesia (25). Rats were allowed several days recovery from surgery and were then placed into an infusion harness and were acclimated to the infusion apparatus for several days during which time they were continuously infused, IP, with sterile 0.9% saline.

In these studies, rats were randomly assigned to either a pentobarbital-infused treatment group or to a saline-infused control group. Rats were then infused for 13 days with either saline or with escalating doses of pentobarbital sodium. Rats were initially infused with pentobarbital at a dose of 100 mg/kg/24 h with subsequent doses being adjusted, daily, according to the degree of CNS depression exhibited by the individual rat. By day 13, rats typically were receiving a dose of 1000 mg/kg/24 h. The escalating dosage schedule and the CNS depression rating scale have been described previously (25). Body weight and water consumption were monitored daily.

On Day 14, there began a 72-hour drug-free period during which all rats were infused with saline. Body weight and water consumption were noted daily. In addition, behavioral signs indicative of withdrawal from barbiturates were noted every hour for the first twelve hours and at 24, 48, and 72 hours. The withdrawal sign rating scale used has been described previously (26). Briefly, the rats are assigned a withdrawal score based on the response of the rat to: 1) a puff of air directed at the head; 2) response to prodding of the flank with a blunt probe; 3) presence of "high" posture characterized as standing on all four feet with an arched back and with or without piloerection; 4) the response of the rat to being grasped and held above the floor of the cage. No attempt was made to observe spontaneous convulsive activity during the drug-free period.

Study 1 was designed to demonstrate the effect of acute administration of diltiazem on the expression of withdrawal signs in pentobarbital-dependent rats. In this study, rats were assigned to one of four groups on Day 14. Group 1 rats (n = 10) were saline-infused control rats and received saline injection, IP, twice daily, at 8:30 a.m. and 8:30 p.m. Rats in Group 2 (n = 10) were control rats which received diltiazem, 25 mg/kg, IP, twice daily. Group 3 rats (n = 9) were pentobarbital-infused rats and were injected with saline, IP, twice daily. Finally, Group 4 rats (n = 11) were pentobarbital-infused and received twice daily injections of diltiazem, 25 mg/kg, IP.

Study 2 was designed to determine the effect of diltiazem, administered twice daily during the pentobarbital infusion phase, on the expression of withdrawal signs during the subsequent PB-free period. In this study, rats were assigned to one of the four treatment groups at the start of the 13-day pentobarbital infusion period. Group 1 rats (n = 6) were saline-infused control rats administered vehicle, IP, twice daily, at 8:30 a.m. and 8:30 p.m. Rats in Group 2 (n = 6) were saline-infused and received twice daily administration of diltiazem, 25 mg/kg, IP. Group 3 consisted of pentobarbital-infused rats (n = 6) treated twice daily with vehicle and Group 4 rats (n = 6) were pentobarbital-infused and were treated twice daily with diltiazem, 25 mg/kg, IP.

Pentobarbital sodium was dissolved in 0.9% saline as a 30 mg/ml stock solution. At the time of infusion the appropriate amount of pentobarbital stock solution was drawn up into a 10 ml syringe and the total volume was brought to 10 ml with saline. Diltiazem hydrochloride (Sigma) was dissolved in 0.9% saline. All solutions were sterilized by filtration through 0.2 micron Metricel filters (Gelman Science Inc.) prior to use in the study.

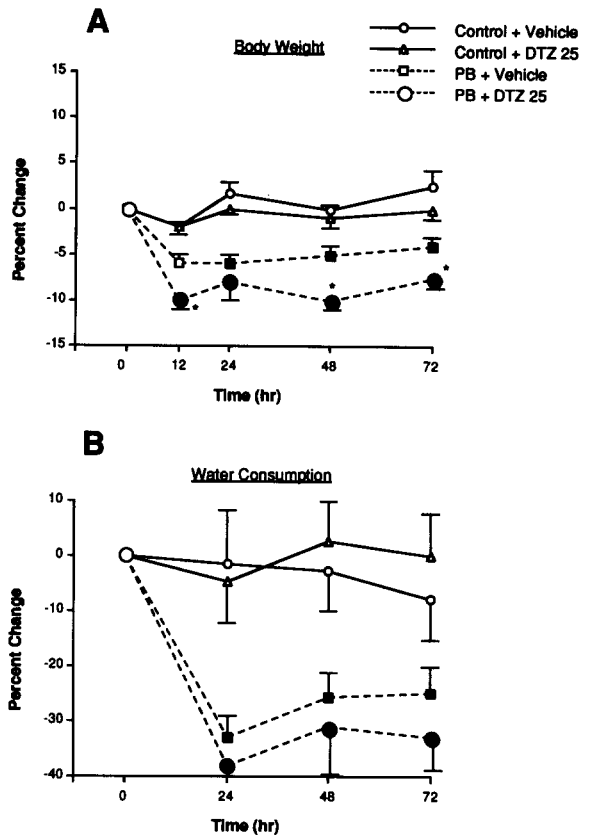

FIG. 1. Percent change in body weight (A) and 24-hour water consumption (B) for control and pentobarbital-dependent rats treated with either vehicle or diltiazem, 25 mg/kg, IP, twice daily, during the 72-hour long withdrawal period. Each point represents the mean percent change, as compared to time zero, for 9–11 rats. Filled symbols indicate significant difference ($p<0.05$) from corresponding control group. *Indicates significant difference ($p<0.05$) between pentobarbital-infused rats treated with vehicle and pentobarbital infused rats treated with diltiazem.

Statistical analysis of withdrawal scores was carried out using the Mann-Whitney U-test. Changes in body weight and water consumption were analyzed using Student's *t*-test.

RESULTS

In both Study 1 and Study 2 the continuous infusion of pentobarbital for 13 days resulted in the establishment of physical dependence on pentobarbital as reflected by decreases in body weight and water consumption (Figs. 1 and 2) and increases in withdrawal scores (Figs. 3 and 4).

In Study 1, it was noted that the body weight of pentobarbital-infused rats treated with saline (Group 3) was depressed by 5% while the body weight of pentobarbital-infused rats treated with diltiazem (Group 4) was depressed by about 10% at 12 hours of withdrawal (Fig. 1A). The body weights continued to be significantly depressed throughout the 72-hour drug withdrawal period. Similarly, the water consumption exhibited by these two groups of rats was significantly depressed by 24 hours into the withdrawal period (Fig. 1B).

Withdrawal scores for Group 3 and Group 4 were noted to be significantly elevated over corresponding control groups by

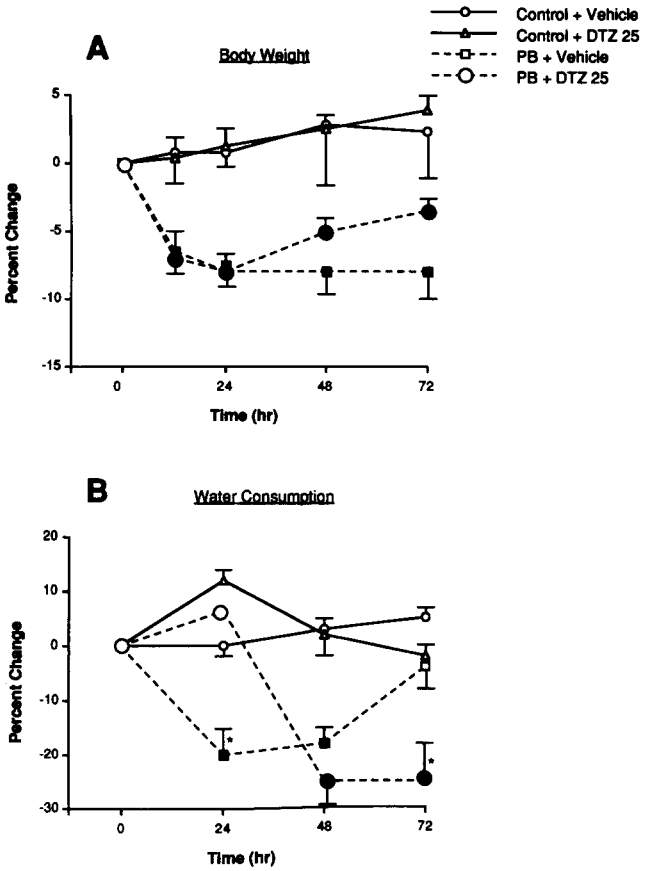

FIG. 2. Percent change in body weight (A) and 24-hour water consumption (B) for control and pentobarbital-dependent rats treated with either vehicle or diltiazem, 25 mg/kg, IP, twice daily, during the 13-day pentobarbital infusion period. Each point represents the mean percent change, as compared to time zero, for 6 rats. Filled symbols indicate significant difference ($p<0.05$) from corresponding control group. *Indicates significant difference ($p<0.05$) between pentobarbital-infused rats treated with vehicle and pentobarbital-infused rats treated with diltiazem.

the fifth hour of the withdrawal period (Fig. 3). There was no significant difference in the withdrawal scores between Group 3 and Group 4 at any time during the withdrawal period.

In Study 2, body weights of pentobarbital-infused rats treated with either vehicle (Group 3) or diltiazem (Group 4) were found to have been significantly decreased during the withdrawal period (Fig. 2A). Interestingly, while the water consumption of rats in Group 3 was found to be significantly depressed by 24 hours of the withdrawal period, the water consumption of the pentobarbital-infused rats treated with diltiazem was not significantly depressed until 48 hours after the start of the withdrawal period (Fig. 2B). At 72 hours, while the water consumption of rats in Group 3 had returned to control levels, the water consumption of rats in Group 4 continued to be significantly depressed.

Pentobarbital-infused rats treated with diltiazem showed significantly elevated withdrawal scores by as early as three hours into the PB-free period and the withdrawal scores continued to remain significantly greater than withdrawal scores of the corresponding control groups up to 48 hours of this period (Fig. 4). Throughout this period the withdrawal scores also tended to be somewhat higher than the withdrawal scores noted for the pentobarbital rats which did not receive diltiazem.

It was also noted that the pentobarbital-dependent rats that were treated daily with diltiazem showed 50% mortality during the withdrawal period. Mortality was not evident in this group during the previous pentobarbital infusion period nor did any deaths occur in any of the other treatment groups.

DISCUSSION

It has been previously demonstrated that the calcium channel blockers may interact with CNS depressants to modify both the acute and chronic effects of these drugs (1, 9, 24). In the current investigation it was demonstrated that diltiazem was able to exacerbate at least some of the typical signs indicative of withdrawal from pentobarbital. This was especially true in Study 2 in which the twice daily administration of diltiazem, along with the continuous infusion of pentobarbital, led to alterations in the pattern of 24-hour water consumption as well as alterations in the intensity of the typical withdrawal signs (Figs. 2B and 4). It

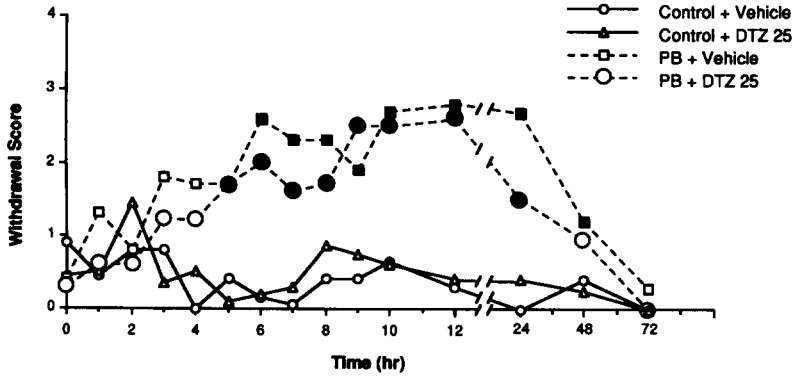

FIG. 3. Mean withdrawal scores for control and pentobarbital-dependent rats treated with either vehicle or diltiazem, 25 mg/kg, IP, twice daily during the 72-hour long withdrawal period. Each point represents the mean withdrawal score of 9–11 rats. Filled symbols indicate significant difference ($p<0.05$) as compared to corresponding control groups.

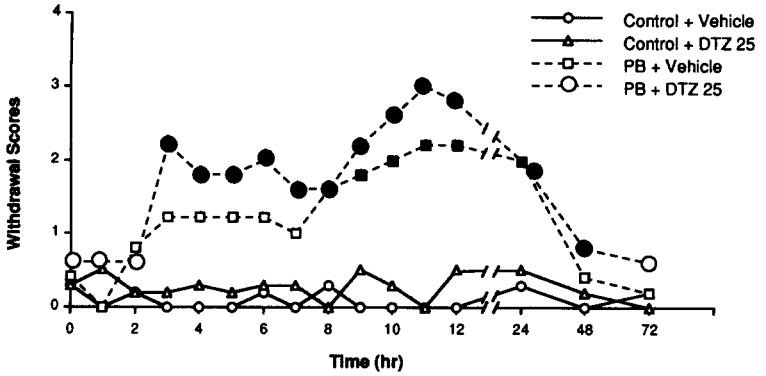

FIG. 4. Mean withdrawal scores for control and pentobarbital-dependent rats treated with either vehicle or diltiazem, 25 mg/kg, IP, twice daily, during the 13-day pentobarbital infusion period. Each point represents the mean withdrawal score of 6 rats. Filled symbols indicate significant difference ($p<0.05$) as compared to corresponding control groups.

appears that treatment of these rats with diltiazem delayed the onset of the hypodipsia by 24 hours and significantly elevated the mean withdrawal scores by as early as 3 hours after the start of the pentobarbital-free period. In control rats, diltiazem administration did not significantly alter any of the parameters observed.

It is difficult to elucidate the nature of the interaction between barbiturates and calcium channel blockers as evidenced by the results of the present study. The data suggest that diltiazem exacerbates at least some of the typical withdrawal symptomology observed in pentobarbital-dependent rats. The fact that this exacerbation of withdrawal signs was apparent even when diltiazem was no longer being administered (i.e., during the PB-free period in Study 2) suggests that the chronic administration of diltiazem may lead to a persistent alteration of the function of the calcium channel.

The evidence from previous studies suggests that the barbiturates, along with other CNS depressant drugs, are capable of inhibiting depolarization-induced calcium conductance as well as altering calcium flux across the neuronal membrane and that tolerance to this effect appears to develop along with the development of tolerance to the effects of barbiturates (4, 7, 12–14, 16, 17, 19, 23). Inhibition of calcium flux at the level of the presynaptic neuronal membrane may lead to inhibition in the release of GABA, or other neurotransmitters, which are ultimately involved in the mediation of the action of barbiturates (27). Importantly, the efflux of GABA from synaptosome preparations has been shown to be inhibited by calcium channel blockers in concentrations which inhibit calcium entry into cells (6).

ACKNOWLEDGEMENTS

This paper was presented, in part, at the fall meeting of the American Society for Pharmacology and Experimental Therapeutics, Milwaukee, WI, August 12–15, 1990. M. Reynen was the recipient of a stipend from the USDSM Summer Medical Student Research Program. This research project was supported, in part, by a grant from the USD General Research Fund and by NIDA grant DA-04244.

REFERENCES

1. Baeyens, J. M.; Del Pozo, E. Interaction between calcium channel blockers and non-cardiovascular drugs. Interaction with drugs acting at the neuromuscular or the CNS level. Pharmacol. Toxicol. 62:59–63; 1988.
2. Barrios, M.; Baeyens, J. M. Differential effects of calcium channel blockers and stimulants on morphine withdrawal in vitro. Eur. J. Pharmacol. 152:175–178; 1988.
3. Bongianni, F.; Carla, V.; Moroni, F.; Pellegrini-Giampietro, D. E. Calcium channel inhibitors suppress the morphine-withdrawal syndrome in rats. Br. J. Pharmacol. 88:561–567; 1986.
4. Carlen, P. L.; Wu, P. H. Calcium and sedative-hypnotic drug action. Int. Rev. Neurobiol. 29:161–189; 1988.
5. Caro, G.; Barrios, M.; Baeyens, J. M. Dose-dependent and stereoselective antagonism by diltiazem of naloxone precipitated morphine abstinence after acute morphine dependence in vivo and in vitro. Life Sci. 43:1523–1527; 1988.
6. Carvalho, C. M.; Santos, S. V.; Carvalho, A. P. Aminobutyric acid release from synaptosomes as influenced by Ca^{+2} and Ca^{+2} channel blockers. Eur. J. Pharmacol. 131:1–12; 1989.
7. Daniell, L. C.; Harris, R. A. Effects of chronic ethanol treatment and selective breeding for hypnotic sensitivity to ethanol on intracellular ionized calcium concentration in synaptosomes. Alcohol.: Clin. Exp. Res. 12:179–183; 1985.
8. Del Pozo, E.; Caro, G.; Baeyens, J. M. Analgesic effects of several calcium channel blockers in mice. Eur. J. Pharmacol. 137:155–160; 1987.
9. Dolin, S. J.; Little, H. J. Augmentation by calcium channel antagonists of general anesthetic potency in mice. Br. J. Pharmacol. 88: 909–914; 1986.
10. Dolin, S. J.; Halsey, M. J.; Little, H. J. Effects of the calcium channel activator, BAY K 8644, on general anesthetic potency in mice. Br. J. Pharmacol. 94:413–422; 1988.
11. Greenberg, D. A. Calcium channels and calcium channel antagonists. Ann. Neurol. 21:317–330; 1987.
12. Gross, R. A.; MacDonald, R. L. Barbiturates and nifedipine have different and selective effects on calcium currents of mouse DRG neurons in culture. Neurology 38:443–451; 1988.
13. Gunderson, C. B.; Umbach, J. A.; Swartz, B. E. Barbiturates depress currents through human brain calcium channels studied in Xenopus oocytes. J. Pharmacol. Exp. Ther. 247:824–829; 1988.
14. Harris, R. A.; Jones, S. B.; Bruno, P.; Bylund, D. B. Effects of dihydropyridine derivatives and anticonvulsant drugs in [^{3}H]-nitrendipine binding and calcium and sodium flux in brain. Biochem. Pharmacol. 34:2187–2191; 1988.
15. Hasegawa, G. R. Nicardipine, nitrendipine and bepridil; new calcium antagonists for cardiovascular disorders. Clin. Pharmacol. 7:97–108; 1988.
16. Leslie, S. W.; Elrod, S. V.; Coleman, R.; Belknap, J. K. Toler-

ance to barbiturates and chlorpromazine induced central nervous system sedation. Involvement of calcium mediated stimulus-secretion coupling. Biochem. Pharmacol. 28:1437–1440; 1979.
17. Marks, S. S.; Watson, D. L.; Carpenter, C. L.; Messing, R. O.; Greenberg, D. A. Calcium channel antagonist receptor in cerebral cortex from alcoholic patients. Brain Res. 487:196–198; 1989.
18. Mendelson, W. B.; Owen, C.; Skolnick, P.; Paul, S. M.; Martin, J. V.; Ko, G.; Wagner, R. Nifedipine blocks sleep induction by flurazepam in the rat. Sleep 7:64–68; 1984.
19. Ondrusek, M. G.; Belknap, J. K.; Leslie, S. W. Effects of acute and chronic barbiturate administration on synaptosomal calcium accumulation. Mol. Pharmacol. 15:386–395; 1979.
20. Piepho, R. W.; Bloedow, D. C.; Lacz, J. P.; Runser, D. J.; Dimmit, D. C.; Browne, R. K. Pharmacokinetics of diltiazem in selected animal species and human beings. Am. J. Cardiol. 49:525–528; 1982.
21. Snyder, S. H. Drug and neurotransmitter receptors: New perspectives with clinical relevance. JAMA 261:3126–3129; 1989.
22. Sutoo, D.; Akiyama, K.; Iimura, K. Effects of calmodulin antagonists on calcium and ethanol-induced sleeping time in mice. Pharmacol. Biochem. Behav. 23:627–631; 1985.
23. Werz, M. A.; MacDonald, R. L. Barbiturates decrease voltage-dependent calcium conductance of mouse neurons in dissociated cell culture. Mol. Pharmacol. 28:269–277; 1985.
24. Wu, P. H.; Pham, T.; Naranjo, C. A. Nifedipine delays the acquisition of tolerance to ethanol. Eur. J. Pharmacol. 139:233–236; 1987.
25. Yutrzenka, G. J.; Patrick, G. A.; Rosenberger, W. Chronic intraperitoneal infusion of pentobarbital: A model of barbiturate dependence in the rat. J. Pharmacol. Exp. Ther. 232:111–118; 1985.
26. Yutrzenka, G. J.; Patrick, G. A.; Rosenberger, W. Substitution of temazepam and midazolam in pentobarbital-dependent rats. Physiol. Behav. 46:55–60; 1989.
27. Zucker, R. S.; Lando, L. Mechanism of transmitter release: Voltage hypothesis and calcium hypothesis. Science 231:574–579; 1986.