Pharmacology Biochemistry and Behavior, Vol. 43, pp. 1263–1267, 1992

0091-3057/92 $5.00 + .00

BRIEF COMMUNICATION

Oxytocin Blocks the Environmentally Conditioned Compensatory Response Present After Tolerance to Ethanol-Induced Hypothermia in Mice

EZIO TIRELLI,*[1] CHRISTINE JODOGNE* AND JEAN-JACQUES LEGROS†

**Laboratoire de Psychologie Expérimentale, Université de Liége au Sart Tilman, Boulevard du Rectorat 5, Bt. 32, B-4000, Liége-1, Belgium*
†Section de Neuroendocrinologie, Université de Liége au Sart Tilman, Centre Hospitalier Universitaire (CHU), Bt. 23, B-4000, Liége-1, Belgium

Received 13 March 1992

TIRELLI, E., C. JODOGNE AND J.-J. LEGROS. *Oxytocin blocks the environmentally conditioned compensatory response present after tolerance to ethanol-induced hypothermia in mice.* PHARMACOL BIOCHEM BEHAV **43**(4) 1263–1267, 1992.—The present study tested the hypothesis that the attenuation by oxytocin of tolerance to ethanol-induced hypothermia relies upon an impairment of the putative conditioning processes underlying environment-specific tolerance. According to the conditioning model of tolerance, such tolerance occurs because an opposite compensatory response conditioned to ethanol-paired cues attenuates ethanol's effects. Tolerance to ethanol-induced hypothermia was established to a particular environment over 4 days by injecting mice (daily) with oxytocin 2 h before ethanol, outside the colony room. As controls, other mice were injected similarly but following testing in the animal room. We found that oxytocin suppressed the conditioned compensatory response, revealed by injecting saline to every group in the tolerance-associated environment. These results suggest that oxytocin acted, at least partly, via an inhibition of the associative learning processes that facilitate tolerance development.

Oxytocin Ethanol Conditioned tolerance Conditioned compensatory response Hypothermia

INCREASING evidence suggests that neurohypophyseal hormones are involved in certain adaptative responses to drug effects. For example, oxytocin influences tolerance to and dependence upon drugs such as ethanol, morphine, and heroin [for reviews, see (2,16)]. Several experiments indicate that oxytocin administered at doses without apparent effect prior to each ethanol injection can inhibit the development of tolerance to ethanol-induced hypothermia and narcosis assessed in a specific environment in rats (26,31,32). Similar results have been reported for the C-terminal fragment of oxytocin prolyl-leucyl-glycinamide [melanocyte-stimulating hormone inhibiting factor-I (MIF-I)], as well as for other fragment analogs [(30); for reviews, see (14,32)]. Repeated injections of oxytocin or MIF-I from the beginning of tolerance development seem to be necessary for its inhibition because when injected acutely after tolerance to the effects of ethanol has developed the peptides are ineffective (9–11,27,31).

It has been proposed that the effects of neurohypophyseal peptides on tolerance are analogous to those separately reported on learning and retrieval processes (3). In the case of oxytocin, the inhibitory effects oxytocin has on tolerance parallel those (also disruptive) it has on learning and memory performances [for review, see (14,15)].

A basis for this proposition can be found in another series of experiments, which have implicated a role for classic conditioning in tolerance to the effects of a variety of drugs in rodents and humans (5,18,21–23,34,35). In particular, tolerance to the hypothermic effects of ethanol have repeatedly been found more pronounced when the drug effect is assessed in the environment in which subjects have previously received

[1] To whom requests for reprints should be addressed.

the drug rather than in an alternative environment. Moreover, under placebo conditions (saline injection) subjects placed in the environment associated with the action of ethanol show hyperthermia, a response opposite in direction to the acute effect of ethanol (8,18,19,35). The contribution of environmental stimuli to tolerance development has been incorporated into an associative model of tolerance (29). This model proposes that, during successive drug administrations, environmental cues become conditioned stimuli (CS) that elicit an adaptative compensatory response. The progressive development of this conditioned response attenuates the initial effect of the drug, so leading to an environment-specific, or conditioned, tolerance [see also (8)].

Thus, it is possible that the effects of oxytocin on tolerance to hypothermia induced by ethanol is exerted (at least partly) via a disruption of the conditioning processes involved in the establishment of tolerance. More specifically, this implies that if the inhibitory effect of oxytocin is due to its disruption of conditioning processes a suppression (at least partial) of the compensatory response will result from the oxytocin treatment. The present study tested this hypothesis.

The compensatory response can be revealed, after tolerance development, by injecting all animals with saline before testing them in the environment associated with the development of tolerance. In the present experiment, mice were still under oxytocin during this test to keep the same internal environment as during tolerance establishment and thereby avoid any effect of suspending oxytocin injection during the test. Indeed, it has been shown that peptides can be conditionally predictive of the effects of a subsequent drug administration. McLaughlin and colleagues (20) found that rats rendered tolerant to morphine (analgesia) in the presence of MIF-I (the C-terminal fragment of oxytocin), at intrinsically inactive doses, exhibited a loss of tolerance when tested without MIF-I, suggesting a CS role for MIF-I.

METHOD

Animals

Thirty-two experimentally naive, random-bred OF-1 male mice, derived from the Carworth-Farms CF-1 strain (IFFA-CREDO, Oncins, France) and born in our laboratory colony, were used at age 12–15 weeks. Mice weighed 34–40 g at the beginning of the experiment. They were individually housed in opaque plastic cages (at least 3 weeks before the experiment) and kept in a colony room on a 12 L : 12 D cycle (lights on at 0700 h) at an ambient temperature 23 ± 2°C. Food and tapwater were available ad lib throughout.

Drugs

Oxytocin (U.C.B.-BIOPRODUCTS, Brussels, Belgium) was dissolved in 0.9% NaCl saline solution immediately prior to use and administered IP at 0.005 mg/0.2 ml per animal. Ethanol was injected by the same route at 2 g/kg so that the volume injected was always 0.01 ml/g body weight. The IP route of administration has been used successfully by Szabó and colleagues (30,31). ICV injections, often used to administer neuropeptides, were not used in this study because these injections are particularly stressful and it has been shown that severe stressors can facilitate the development of tolerance to the hypothermic effect of ethanol in rodents (24).

Design and Procedure

The experiment consisted of two phases: first, establishing the effects of oxytocin on tolerance over a period of 4 days; second, testing for the possible effects of oxytocin on the conditioned compensatory response on day 5. Tolerance was established by placing mice into one of the two following groups: mice receiving ethanol paired with the testing environment (referred to as paired group) and mice receiving the same injection of ethanol in the colony room, which was different from the testing room (referred to as unpaired group). To assess the effect of oxytocin, additional mice were divided into two other groups, paired or unpaired as well, in which mice were injected with oxytocin before any other treatment.

During the tolerance establishment phase, four groups of mice (n = 7 or 8; initially n = 8, but two animals died during the experiment) received two IP injections (2 h apart), at approximately the same time each day, and two further IP injections (2 h apart as well) 2 h later. Mice in the two paired groups were given saline plus ethanol, or oxytocin plus ethanol in the experimental room, and two saline injections in the colony room after testing (SAL + ETH and OXY + ETH paired groups). The unpaired groups received two saline injections in the experimental room and saline plus ethanol or oxytocin plus ethanol in the colony room (SAL + ETH and OXY + ETH unpaired groups). The dose of oxytocin was selected on the basis of a preliminary study such that it does not influence the hypothermic effect of ethanol after an acute injection while remaining physiologically active. In addition, in a separate experiment carried out using exactly the same conditions as those of the present study (12) and on other occasions we found that oxytocin at that dose does not change rectal temperature when injected daily for 5 days. Thus, to reduce the practical load no group given oxytocin alone was included here.

Every day, mice were weighed and brought into the experimental room (a small, sound-attenuating, white room illuminated by one 60-watt light bulb and maintained at 21 ± 1°C), where they received the two first injections 2 h apart. All testing was between 1300 and 1800 h. Hypothermia was evaluated by rectal temperature. All measurements were monitored for 20 min before and 20 min after ethanol injection. To graph the compensatory response (less than 1°C) with clarity, the difference between both trials was taken as the measure of thermic response. There were no changes in the preinjection temperatures, neither across days nor between groups. A small lubricated thermocouple connected to an electronic thermometer (RS Components, Bristol, United Kingdom; No. 610-067) was used as the probe. The animal was loosely restrained on a wire mesh grid and the probe was inserted 1.5 cm into the rectum for approximately 30 s, until the temperature stabilized. Because pilot experiments suggested that some irritation may appear after six daily probe insertions into the rectum, the duration of the experiment was limited to five daily tests. After the second temperature measurement, mice remained for 60 min in the testing room. At the end of this period, they were returned to the colony room and received the two last injections 2 h apart. In fact, it is likely that the putative conditioned stimuli were mostly provided by the handling cues during temperature assessments and injections.

On the conditioned compensatory response test day (day 5), paired as well as unpaired groups received saline in the testing environment after having received saline (SAL + ETH groups) or oxytocin (OXY + ETH groups) injections.

Statistical Analysis

To ensure that oxytocin significantly attenuated tolerance to ethanol-induced hypothermia, temperature measurements taken during tolerance establishment for the paired groups were submitted to a mixed analysis of variance (ANOVA), with oxytocin (oxytocin vs. saline) considered a between-group factor and test day (1–4) a within-subject variable (13). To adjust for potential violations of the assumptions of compound symmetry and sphericity, the Greenhouse-Geisser correction was also computed. Data from the test day (day 5) were treated with a two-way ANOVA having environment (two levels) and oxytocin (two levels) as crossed factors. Spjøtvoll-Stoline's tests (unequal cells), derived from the appropriate error mean squares, were used subsequently for comparing saline- to oxytocin-pretreated groups and paired with unpaired groups.

RESULTS

As shown in the left panel of Fig. 1, an attenuative effect of oxytocin was observed (OXY + ETH paired group) as soon as tolerance to ethanol convincingly emerged (SAL + ETH paired group), on the third day of pretreatment. The responses of the OXY + ETH paired group on days 2–4 were not significantly lower than on day 1. Note that on the first test day oxytocin (administered 2 h before ethanol) was without effect on ethanol-induced hypothermia. This profile was supported by a robust interaction between oxytocin and day and the appropriate pairwise comparisons, $F(3, 39) = 5.08$, $p < 0.0046$. Despite the adjustment provided by the Greenhouse-Geisser correction, this effect remained highly significant (thus, for clarity, the original degrees of freedom and F were given). This relation was also seen in our previous results (12).

The results of the conditioning test are shown in the right panel of Fig. 1. ANOVA revealed a main effect of environment, $F(1, 26) = 5.70$, $p < 0.0246$, and a significant interaction between oxytocin and environment, $F(1, 26) = 5.61$, $p < 0.0256$. Pairwise comparisons indicated that mice in the SAL + ETH paired group, exposed to environmental cues previously associated with ethanol, displayed a clear increase in temperature when compared to the SAL + ETH unpaired group. Therefore, exposure to environmental cues previously associated with ethanol elicits in undrugged mice a thermic response opposite that induced by acute ethanol treatment. This conditioned hyperthermia was suppressed by oxytocin pretreatment: The OXY + ETH paired group showed no significative temperature change and did not differ from the un-

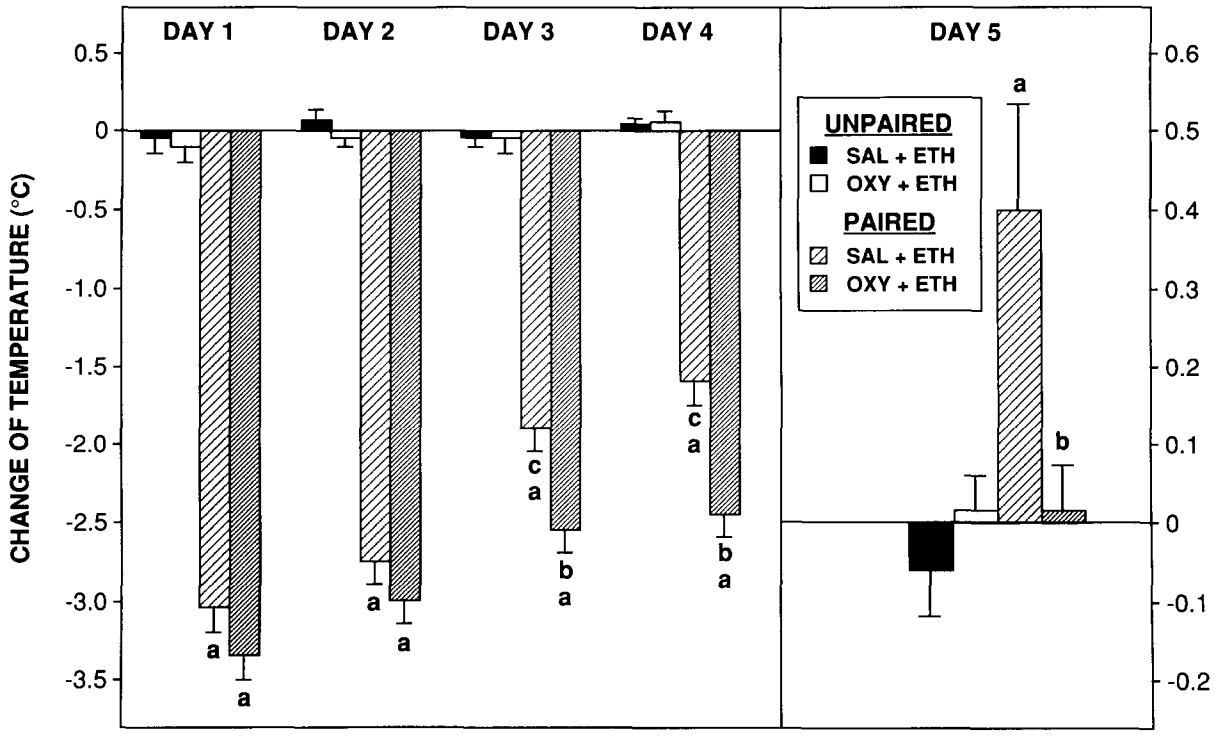

FIG. 1. Effect of oxytocin on the compensatory hyperthermia response conditioned to environmental cues previously associated to ethanol's effect. Over 4 pretreatment days, mice of the two paired groups received saline plus ethanol or oxytocin plus ethanol, 2 h apart, in the experimental room (where temperature measurement was performed) before testing, and two fold saline, 2 h apart, in the colony room after testing. The two unpaired groups were given the same treatment but in the reverse order. The left panel shows the effect of oxytocin on the development of tolerance. On the conditioning test day (day 5, right panel), paired and unpaired groups received saline (SAL+ETH groups) or oxytocin (OXY+ETH groups) plus saline in the testing room. The difference of temperature monitored 20 min prior to and after ethanol injections was used as the measure of hypothermia. Oxytocin and ethanol were injected intraperitoneally at 0.005 mg/animal and 2 g/kg, respectively. Vertical brackets represent SE. (a), significantly different from SAL+ETH and OXY+ETH unpaired groups; (b), different from the SAL+ETH paired group; (c), different from the SAL+ETH paired group mean on day 1; all at $p < 0.01$ (Spjøtvoll-Stoline tests).

paired groups. Examination of the unpaired groups indicated that the body temperature was not affected by 0.005 mg/animal oxytocin injected 2 h before ethanol, confirming our previous results (12).

DISCUSSION

The present results indicate that oxytocin given prior to each ethanol injection impedes not only the full development of tolerance established in a specific environment to ethanol-induced hypothermia but also, and especially, the expression of the compensatory hyperthermia. The facts that our dose of oxytocin did not alter ethanol-induced hypothermia when injected acutely (on day 1) and remained without thermic effect when injected five times daily (12) supports again some specificity of the attenuating effect of oxytocin on the conditioning processes involved in tolerance establishment (in which oxytocin receptors are probably involved).

A number of studies have shown that a drug repeatedly injected before a drug to whose effects tolerance is established may serve as CS for these effects (33). However, it seems unlikely that the oxytocin injections in our experiment served as conditioned stimuli for ethanol tolerance, thereby facilitating the acquisition of the compensatory response. If this were so, the compensatory response would not have been suppressed during the placebo test. Rather, a stronger compensatory response should have occurred.

According to the associative model of tolerance (8), environmental specificity of tolerance to ethanol-induced hypothermia occurs because a compensatory hyperthermia becomes conditioned to the environmental cues signalling the drug effect. Hence, if the inhibitory action of oxytocin on environment-specific tolerance is due to its disruption of conditioning processes a diminution of the compensatory response should occur as well. That is exactly what we found in the present study. The idea that learning may be an important basis for the effects of neuropeptides on tolerance (in particular to morphine and ethanol) and that this can be analogously considered a kind of memory-related phenomenon is not new (3,4,17). However, there is a paucity of studies specifically designed to address this issue, and the present results are to our knowledge the first to directly support this idea. To the extent that environment-specific tolerance to hypothermia and compensatory hyperthermia were generated (at least partly) by associative learning processes, our procedure may be considered akin to the tests used to evaluate the influence of oxytocin on learning and memory processes in rodents (15). Oxytocin has been found to facilitate extinction and attenuate retention of learned responses in conditioned avoidance-based procedures [(1,7,28); for reviews, see (14,15)]. Recently, a comparable effect has been shown in rats that were required to recognize a previously met juvenile after a given retention interval (6,25). In addition, antisera/antagonists against oxytocin attenuated or blocked the disruptive effect of oxytocin in all these procedures (15,25), suggesting the involvement of oxytocin receptors. The present data generalize these effects to another situation and thereby indirectly support the theory of an involvement of an endogenous form of oxytocin in learning and memory processes. Vasopressin, another neurohypophyseal peptide, has the opposite effect on learning and memory from oxytocin (1,7,14,15,17,28). Therefore, to see to what extent the effects of peptides on tolerance can be considered analogous to those on learning and memory future research will be devoted to comparing the effects of these two neuropeptides on the compensatory response and manipulating their effects with antisera/antagonists.

ACKNOWLEDGEMENTS

The authors thank Philip Terry and Stephen T. Tiffany for their valuable comments and corrections.

REFERENCES

1. Bohus, B.; Kovacs, G. L.; De Wied, D. Oxytocin, vasopressin and memory: Opposite effects on consolidation and retrieval processes. Brain Res. 157:414–417; 1980.
2. Colbern, D. L.; Ritzmann, R. F.; Krivoy, W. Neurohypophyseal peptides in tolerance and dependence. In: De Wied, D.; Gispen, W. H.; Van Wimersma Greidanus, T. B., eds. Neuropeptides and behavior. vol. 2. The neurohypophyseal hormones. Oxford, UK: Pergamon Press; 1986:171–186.
3. Crabbe, J. C.; Rigter, H. Learning and the development of alcohol tolerance and dependence: The role of vasopressin-like peptides. Trends Neurosci. 1:20–23; 1980.
4. Crabbe, J. C.; Rigter, H.; Kerbush, S. Genetic analysis of tolerance to ethanol hypothermia in recombinant inbred mice: Effect of desglycinamide (9)-arginine (8)-vasopressin. Behav. Gen. 10: 139–153; 1980.
5. Dafters, R.; Anderson, G. Conditioned tolerance to the tachycardia effect of ethanol in humans. Psychopharmacology (Berl.) 78: 365–367; 1982.
6. Dantzer, R.; Bluhé, R. M.; Koob, G. F.; Le Moal, M. Modulation of social memory in male rats by neurohypophyseal peptides. Psychopharmacology (Berl.) 91:363–368; 1987.
7. De Wied, D.; Gaffori, O.; Burbach, J. P. H.; Kovács, L.; Van Ree, J. Structure–activity relationships studies with C-terminal fragments of vasopressin and oxytocin on avoidance behaviours of rats. J. Exp. Pharmacol. Ther. 241:268–274; 1987.
8. Hinson, R. E.; Siegel, S. The contribution of Pavlovian conditioning to ethanol tolerance and dependence. In: Rigter, H.; Crabbe, J. C., eds. Alcohol tolerance and dependence. Amsterdam: Elsevier/North Holland Biomedical Press; 1980:181–199.
9. Hoffman, P. L. Structural requirements for neurohypophyseal peptide maintenance of ethanol tolerance. Pharmacol. Biochem. Behav. 17:685–690; 1982.
10. Hoffman, P. L.; Ritzmann, R. F.; Tabakoff, B. The influence of arginine-vasopressin and oxytocin on ethanol dependence and tolerance. In: Galanter, M., ed. Currents in alcoholism. vol. 5. New York: Grune & Stratton; 1980:5–16.
11. Hoffman, P. L.; Ritzmann, R. F.; Walter, R.; Tabakoff, B. Arginine-vasopressin maintains ethanol tolerance. Nature 276: 614–616; 1976.
12. Jodogne, C.; Tirelli, E.; Klingbiel, P.; Legros, J.-J. Oxytocin attenuates tolerance not only to the hypothermic but also to the myorelaxant and akinesic effects of ethanol in mice. Pharmacol. Biochem. Behav. 40:261–265; 1991.
13. Kirk, R. E. Experimental design: Procedures for the behavioral sciences. Belmont, CA: Brooks/Cole; 1982.
14. Kovács, G. L.; Szabó, G.; Sarnayi, Z.; Telegdy, G. Neurohypophyseal hormones and behavior. In: De Kloet, E. R.; Wiegant, V. M.; De Wied, D., eds. Progress in brain research. vol. 76. Amsterdam: Elsevier Science Publishers; 1987:109–117.
15. Kovács, G. L.; Telegdy, G. Role of oxytocin in memory processes and amnesia. In: De Wied, D.; Gispen, G. W.; van Wimersma Greidanus, T. B., eds. Neuropeptides and behavior. vol. 2. The neurohypophyseal hormones. Oxford, UK: Pergamon Press; 1986:123–140.
16. Kovács, G. L.; Telegdy, G. Neurohypophyseal peptides, moti-

vated and drug-induced behaviour. In: Telegdy, G., ed. Frontiers of hormone research. vol. 15. Neuropeptides and brain function. Basel: Karger; 1987:138–174.
17. Krivoy, W. A.; Zimmermann, E.; Lande, S. Facilitation of development to resistance to morphine analgesia by desglycinamide (9)-lysine vasopressin. Proc. Natl. Acad. Sci. USA 71:1852–1856; 1974.
18. Lê, A. D.; Poulos, C. X.; Cappell, A. Conditioned tolerance to the hypothermic effect of ethyl alcohol. Science 206:1109–1110; 1979.
19. Mansfield, F. G.; Cunningham, C. L. Conditioning and extinction of tolerance of the hypothermic effect of ethanol in rats. J. Comp. Physiol. Psychol. 94:962–969; 1980.
20. McLaughlin, C. R.; Lichtman, A. H.; Fanselow, M. S.; Cramer, C. P. Pro-Leu-Gly-NH2 serves as a conditioned stimulus in the acquisition of conditioned tolerance. Behav. Neurosci. 103:447–451; 1989.
21. Melchior, C. L. Environment-dependent tolerance to ethanol produced by intracerebroventricular injections in mice. Psychopharmacology (Berl.) 96:258–261; 1988.
22. Melchior, C. L.; Tabakoff, B. Modification of environmentally cued tolerance to ethanol in mice. J. Exp. Pharmacol. Ther. 219: 175–180; 1981.
23. Newlin, D. B. Human conditioned compensatory response to alcohol cues: Initial evidence. Alcohol 2:507–509; 1985.
24. Peris, J.; Cunningham, C. L. Stress enhances the development of tolerance to the hypothermic effect of ethanol. Alcohol Drug. Res. 7:187–193; 1987.
25. Popik, P.; Vetulani, J. Opposite action of oxytocin and its peptide antagonists on social memory in rats. Neuropeptides 18:23–27; 1991.
26. Pucilowski, O.; Kostowski, W.; Trzaskowa, E. The effect of oxytocin and fragment (MIF-I) on the development of tolerance to hypothermic and hypnotic action of ethanol in the rat. Peptides 6:7–10; 1985.
27. Rigter, H.; Crabbe, J. C. Alcohol: Modulation of tolerance by neuropeptides. In: Sandler, M., ed. Psychopharmacology of alcohol. New York: Raven Press; 1980:179–189.
28. Schulz, H.; Kovács, G. L.; Telegdy, G. Effect of physiological doses of vasopressin and oxytocin on avoidance and exploratory behavior in rats. Acta Physiol. Hung. 45:211–215; 1974.
29. Siegel, S. Evidence from rats that morphine tolerance is a learned response. J. Comp. Physiol. Psychol. 89:498–506; 1975.
30. Szabó, G.; Kovács, G. L.; Székeli, S.; Baláspiri, L.; Telegdy, G. C-Terminal fragments of oxytocin (propyl-leucyl-glycinamide and Z-propyl-D-leucine) attenuate the development of tolerance to ethanol. Acta Physiol. Hung. 69:115–122; 1987.
31. Szabó, G.; Kovács, G. L.; Székeli, S.; Telegdy, G. The effects of neurohypophyseal hormones on tolerance to the hypothermic effect of ethanol. Alcohol 2:567–574; 1985.
32. Szabó, G.; Kovács, G. L.; Telegdy, G. Neurohypophyseal peptides and ethanol tolerance and dependence. In: Telegdy, G., ed. Frontiers of hormone research. vol. 15. Neuropeptides and brain function. Basel: Karger; 1987:128–137.
33. Taukulis, H. K. Conditional hyperthermia in response to atropine associated with a hypothermic drug. Psychopharmacology (Berl.) 90:327–331; 1986.
34. Tiffany, S. T.; McCal, K. L.; Maude-Griffin, P. M. The contribution of classical conditioning to tolerance to the antinociceptive effects of ethanol. Psychopharmacology (Berl.) 92:524–528; 1987.
35. Zinatelli, M.; Vogel-Sprott, M. Learned tolerance to alcohol: Mental rehearsal with imagined consequences. Alcohol. Clin. Exp. Res. 14:518–521; 1990.