Pharmacology Biochemistry and Behavior, Vol. 46, pp. 309–314, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Decreased Tolerance to Ethanol-Induced Hypothermia in Long-Term Castrate Male Rats

ROBERT F. McGIVERN,*[1] TED MELCER* AND CHRISTINE L. MELCHIOR†

*Department of Psychology, San Diego State University, 6363 Alavarado Court, Suite 207, San Diego, CA 92120
†Brentwood Division Research, West Los Angeles Veterans Administration Medical Center, Los Angeles, CA 90073*

Received 9 September 1992

McGIVERN, R. F., T. MELCER AND C. L. MELCHIOR. *Decreased tolerance to ethanol-induced hypothermia in long-term castrate male rats.* PHARMACOL BIOCHEM BEHAV 46(2) 309–314, 1993.—A potential role for central stores of vasopressin in the development of tolerance was studied in the long-term castrate rat. Vasopressin stores in the septal region are known to be dramatically depressed following long-term castration. Sprague–Dawley male rat littermates were castrated at 26 days of age or given a sham surgery. Experiments began when animals reached 130 days of age. Tolerance to the hypothermic effects of ethanol occurred in intact but not castrate animals over the course of six daily IP injections of 3.0 g/kg ethanol. Both groups exhibited tolerance to the length of time needed to return to baseline temperature over the 6 days of ethanol injections. Tolerance to this effect of ethanol was still evident in intact animals but not castrates following another injection of ethanol 1 week later. No tolerance developed to the rebound hyperthermia that occurred in both groups. Blood ethanol levels did not differ significantly between castrate and intact littermates administered a single dose of ethanol. Overall, these results support the hypothesis that endogenous vasopressin is involved in the development of some aspects of tolerance to ethanol.

Vasopressin Castration Ethanol Hypothermia Rats Tolerance

THE administration of vasopressin has been shown to influence both the acquisition and maintenance of tolerance to ethanol in rodents [see (13) for review]. Acquisition of tolerance to ethanol is blocked or delayed when vasopressin is administered (4,19,20). On the other hand, daily injections of vasopressin or nonpressor analogs following cessation of ethanol administration will maintain tolerance for extended periods (13,14,17). Use of vasopressin agonists and antagonists that are relatively selective for different receptor subtypes has shown that this effect of vasopressin is mediated by V_1 receptors in the CNS (27).

The septum has a high density of V_1 receptors, suggesting that this area may be important for the effect of vasopressin on tolerance to ethanol (11). In the present experiments, this possibility was explored by determining the effects of reducing septal vasopressin synthesis on tolerance to ethanol-induced hypothermia. Vasopressin synthesis in this region is regulated by circulating androgens. Neuronal synthesis of vasopressin in the bed nucleus of stria terminalis (BNST), whose projections densely innervate the lateral septum, is stimulated by circulating estrogens converted from androgens (8). Vasopressin synthesis in the septal pathway (5) is therefore dramatically reduced following long-term (10–15 weeks) castration (5,7,8). This effect is reversed by administration of testosterone (7). While vasopressin levels are severely reduced, the binding of vasopressin is not altered by castration (28).

In choosing to utilize castration as a means of reducing vasopressin levels, some caution must be exercised because other features of this preparation might effect the response to ethanol. Castrates catabolize ethanol more quickly than controls, as indicated by elevated levels of alcohol dehydrogenase (ADH) (2,3), but neither the rising nor peak levels of ethanol in the blood are altered by castration. Given this pattern of ethanol metabolism, the maximal hypothermic response to ethanol in castrates should not be different from intact animals but castrates could return to baseline body temperature more rapidly than controls.

Another phenomena that has been demonstrated in castrates is an altered thermoregulatory response to elevated body temperature. Castration as a means of depleting septal vasopressin levels has been used to substantiate the role of this pathway in the modulation of hyperthermia (16,24). Castrated rats demonstrate an exaggerated fever in response to administration of prostaglandins. Although castrated rats maintain

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

body temperatures no different from controls in ambient temperatures of 24 or 9°C, they display a much higher temperature than controls at 32°C (24). While injection of ethanol at room temperature causes hypothermia, Gallaher and Egner (10) reported that a rebound hyperthermia follows. Castrates may therefore demonstrate an enhanced rebound hyperthermia.

The experimental design chosen for this study minimized the potential contribution of other factors to the response to ethanol or development of tolerance that might differentially affect castrates. Peris and Cunningham (23) demonstrated that stress can exacerbate the hypothermic response to ethanol and influence the development of tolerance. In this experiment, the potential stress of a novel environment or handling was minimized by giving animals several days to acclimate to the test environment and administering saline injections prior to the first injection of ethanol. Previous exposure to these stimuli, as well as the close spacing of a relatively high dose of ethanol, minimized a contribution by learning factors to the development of tolerance (1,26).

METHOD

Sprague-Dawley male littermates (Charles River, Portage, MI) born in our laboratory were castrated at 26 days of age or given a sham surgery under ketamine/xylazine anesthesia. Littermates were represented in both groups. Animals were group housed until the age of testing in a climate-controlled vivarium room. The lighting schedule was 12 L : 12 D with lights on at 0600 h. Dry food pellets (Teklab) and tapwater were available ad lib.

At 130 days of age, castrate and intact animals were implanted with temperature transmitters (Mini-Mitter, Sun River, OR) in the peritoneal cavity. Two weeks later, animals were housed in individual cages in a separate vivarium room and core temperature measurements monitored every 5 min for the remainder of the experiment.

Animals were acclimated to the single cages for 4 days. Beginning on the fifth day, each animal was injected IP with saline for 3 days in an amount equivalent to the later ethanol injections. On the day following the third injection of saline, animals were given IP injections of 3.0 g/kg ethanol (15% v/v in distilled water) daily for 6 successive days. This was followed by 6 days of saline injections and then a seventh injection of ethanol. All animals were tested at the same time. Injections were given at the same time each day (1000 h).

Experimentally naive littermates of animals used in the tolerance experiments described above were used for blood alcohol clearance rates. At 150 days of age, four intact and five castrate males were implanted with jugular cannulae according to procedures we published previously (9). Animals were allowed 4 days for recovery, whereupon they were injected at 1000 h with ethanol (3.0 g/kg ethanol, 15% v/v in distilled water) and returned to their home cage. Blood samples (100 μl) were drawn at 30, 60, 120, 180, 240, and 360 min postinjection, transferred to capped plastic microfuge vials, and placed on ice. Samples were spun down in a refrigerated centrifuge and the serum frozen at −70°C for later assay using a quantitative enzymatic procedure with standards and reagents supplied by a kit (Cat. A-7011) from Sigma Chemical Co. (St. Louis, MO).

Data Analysis

A baseline temperature was established each day for each animal by taking the average temperature for the period between 0900 and 0945 h. Rebound hyperthermia was assessed by subtracting the mean baseline response following saline injections from the baseline response each day following ethanol. Data were analyzed by one-way repeated-measures analysis of variance (ANOVA).

The hypothermic response was measured by taking the lowest temperature recorded during the 4 h following injection. Data were collapsed into 15-min time blocks. Tolerance was assessed in two ways: a) comparing the lowest temperature in

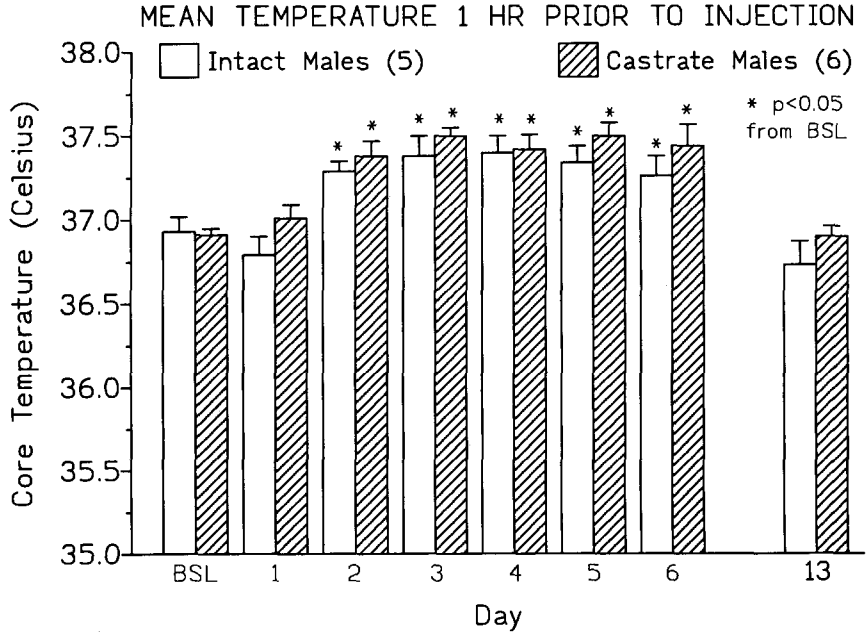

FIG. 1. Mean temperature 1 h prior to ethanol injection in intact or castrate male rats.

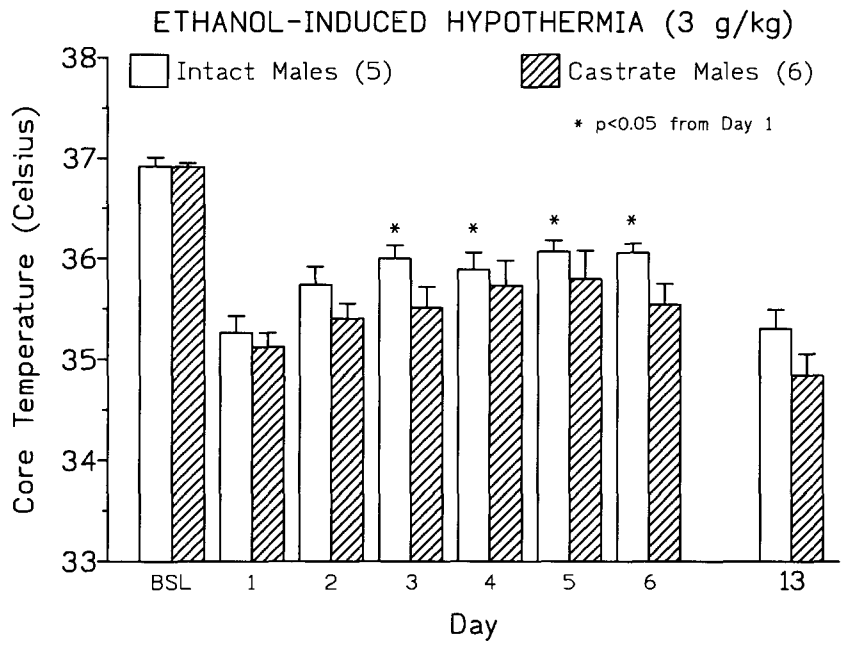

FIG. 2. Hypothermia induced by 3.0 g/kg ethanol injected daily for 6 days and again 1 week later (day 13) in intact and castrate male rats.

response to the first injection of ethanol from the response on each succeeding day; and b) analyzing the change in temperature of the ethanol response from the baseline temperature each day. Planned comparisons for measuring tolerance to hypothermia were conducted using Student's *t*-test.

Time to recovery from hypothermia was determined by measuring the postinjection time when body temperature was above the baseline temperature for three successive 5-min measurements. Determinations were done by two experimenters blind to the hormonal state of animals. These data, as well as blood alcohol data, were also analyzed using a one-way ANOVA with repeated measures.

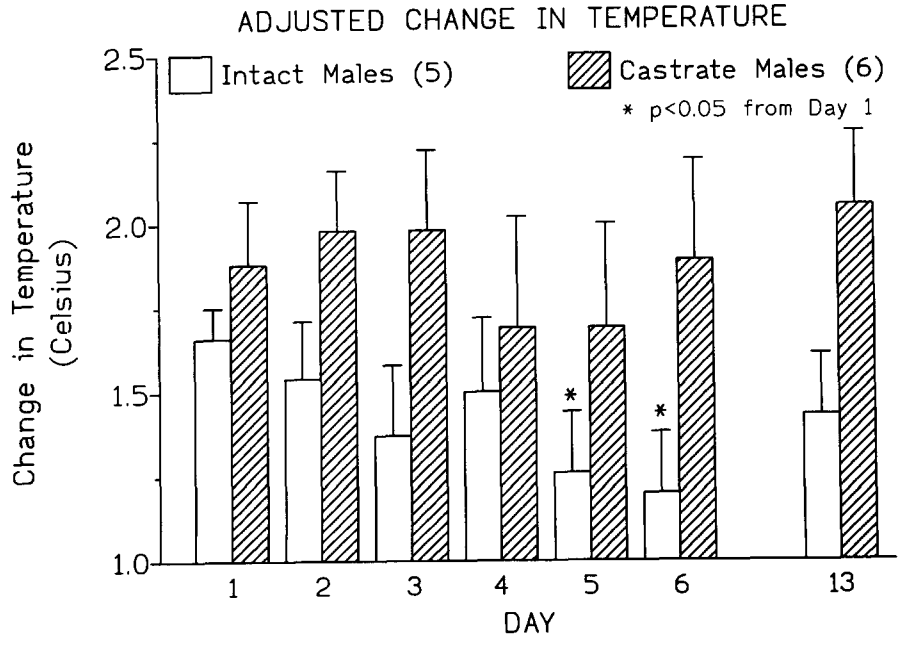

FIG. 3. Change in temperature from the baseline recorded prior to injection to the maximum hypothermia produced by 3.0 g/kg ethanol each day in intact and castrate male rats.

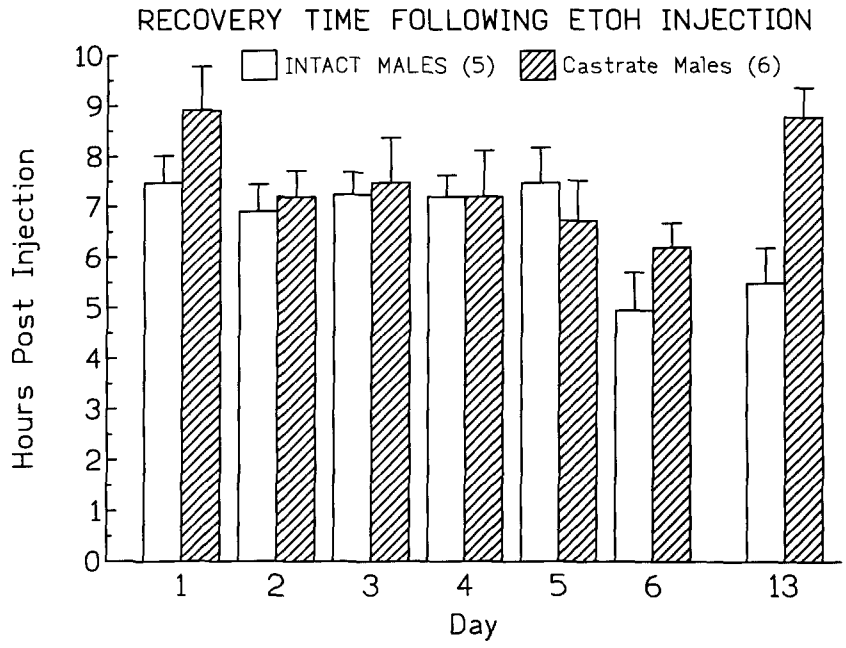

FIG. 4. Time to return to baseline body temperature following injection of 3.0 g/kg ethanol each day in intact and castrate male rats.

RESULTS

Baseline temperature did not differ between castrate and intact animals. The average baseline temperature for the 3 saline days was identical to the average temperature for the 3 preceding uninjected days, indicating that the injection procedure or volume had no effect on baseline temperature. As shown in Fig. 1, baseline temperature following ethanol injection showed a significant rise over days in both groups. The analysis revealed a significant effect for days, $F(5, 45) = 33.24, p < 0.001$, but no effect of castration or an interaction of castration with day. A significant rise in baseline temperature was detected in both groups by the second day following the first ethanol injection when compared to the average saline baseline temperature ($p < 0.05$). This rebound hyperthermia persisted through the sixth day of ethanol injections but was not evident after the seventh ethanol injection on day 13 of the experiment.

When tolerance was assessed by comparing the temperature response to the first ethanol injection with each succeeding response, tolerance was evident in intact animals following the third injection and persisted through the sixth injection but was not evident following the seventh injection 1 week later. No tolerance was observed in castrate animals. Data are shown in Fig. 2.

When tolerance was assessed by the amount of change in temperature of the response to ethanol from the baseline temperature preceding the ethanol injection on each day, tolerance was observed after injections 5 and 6 for intact animals. No tolerance was observed in castrates. Data are shown in Fig. 3.

Figure 4 shows the amount of time to return to baseline temperature for the two groups following each injection of ethanol. The latency to return to baseline decreased following the first six injections for both groups [days, $F(5, 45) = 5.37$, $p < 0.001$], with no significant differences between groups being detected. However, when the recovery time following the seventh injection of ethanol was compared to the time following the sixth injection a significant increase was observed in castrate animals, $t(9) = 2.33$, $p < 0.05$, but not in intact animals.

As shown in Fig. 5, there were no differences between castrate and intact animals in blood alcohol levels at any of the times at which they were measured.

DISCUSSION

Tolerance to ethanol-induced hypothermia was observed in gonadally intact males following six daily IP injections but

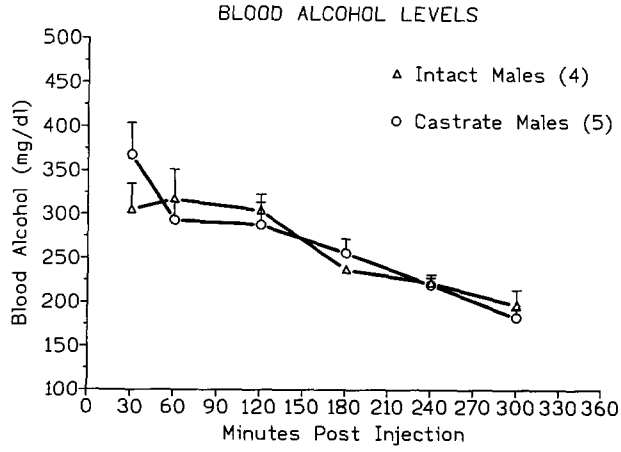

FIG. 5. Blood alcohol levels at various times after injection of 3.0 g/kg ethanol in intact and castrate male rats.

not in long-term castrates. Castrate animals failed to exhibit a hypothermic response on days 2–6 that was significantly less than the response observed following the first injection. However, tolerance to ethanol was observed in both castrates and intact animals in the time to return to baseline temperature. Equal tolerance to this effect was observed in both groups during the acquisition phase of the experiment, but when tolerance to this measure was assessed 1 week after the sixth daily injection only intact animals displayed evidence of tolerance. Overall, these findings support the hypothesis that endogenous vasopressin may facilitate the development and maintenance of tolerance to ethanol.

The finding that castrates fail to develop tolerance is in agreement with the few other studies on the contribution of endogenous vasopressin to the acquisition and maintenance of tolerance to ethanol. Pittman et al. (25) studied the development of tolerance in Brattleboro rats. They found that tolerance did not develop in either the homozygotes that lack vasopressin or the heterozygotes that have low levels of vasopressin. Circumstantial evidence of a role for endogenous vasopressin in the acquisition of tolerance can be inferred from the results of a study by Linkola et al. (18). In that study, a selectively bred alcohol-preferring (AA) strain of rats was found to display significantly more tolerance to alcohol than alcohol-nonpreferring (ANA) rats. AA rats also secreted twice as much pituitary vasopressin as ANA rats in response to a challenge dose of ethanol. More recently, Gulya et al. (1991) reported that chronic ethanol ingestion, which produced functional tolerance in mice, resulted in decreased vasopressin mRNA levels in all areas of the brain examined, including the bed nucleus of the stria terminalis. The authors suggest that this may represent a downregulation of synthesis that could help maintain constant levels of vasopressin. Additional evidence supporting a role for endogenous vasopressin in the maintenance of tolerance was supplied by Szabo et al. (27) by showing that administration of a V_1-selective vasopressin antagonist increased the rate of loss of functional ethanol tolerance.

Rebound hyperthermia was evident in both groups on the day following the second injection of ethanol. The elevation in baseline temperature during this time of day was similar in both groups and remained for the following 5 days. No evidence of tolerance to this effect was observed in either group during the period of daily injections. This rebound hyperthermia effect was no longer evident prior to a single ethanol injection 1 week later. The lack of difference between castrate and intact animals may be attributed to the possibility that the temperatures reached during the rebound hyperthermia were not high enough to involve or differentiate an involvement of the vasopressin system in modulating the hyperthermia.

We did not observe any difference in blood alcohol levels between castrates and intact littermates. This finding is similar to that of Cicero et al. (3), who observed no difference in blood alcohol levels between castrate and intact males 3 h following one of several doses of ethanol. Similarly, others found no difference between castrates and intacts on blood alcohol levels on the ascending and plateau portions of the blood alcohol curve (1,15,21).

In conclusion, castrates do not develop tolerance to the hypothermic actions of ethanol. On the one aspect of ethanol-induced hypothermia in which they did not differ from intact animals on development of tolerance, time to return to baseline, they did not maintain tolerance as intact animals did. These data support the hypothesis that endogenous septal vasopressin is important for the development and maintenance of tolerance to ethanol.

ACKNOWLEDGEMENTS

Expert technical assistance was provided by Catherine Dean. This work was supported in part by a grant from NIAAA (AA06478) and the Veterans Administration.

REFERENCES

1. Baker, T. B.; Tiffany, S. T. Morphine tolerance as habituation. Psychol. Rev. 92:78–108; 1985.
2. Cicero, T. J.; Bernard, J. D.; Newman, K. Effects of castration and chronic morphine administration on liver alcohol dehydrogenase and the metabolism of ethanol in the male Sprague–Dawley rat. J. Pharmacol. Exp. Ther. 215:317–324; 1980.
3. Cicero, T. J.; Greenwald, J.; Nock, B., O'Connor, L. Castration-induced changes in the response of the hypothalamic–pituitary axis to alcohol in the male rat. J. Pharmacol. Exp. Ther. 252: 456–461; 1990.
4. Crabbe, J. C.; Rigter, H.; Kerbusch, S. Genetic analysis of tolerance to ethanol hypothermia in recombinant inbred mice: Effect of desglycinamide(9)-arginine(8)-vasopressin. Behav. Gen. 10: 139–152; 1980.
5. De Vries, G. J.; Best; W.; Sluiter, A. A. The influences of androgens on the development of a sex difference in the vasopressinergic innervation of the rat lateral septum. Dev. Brain Res. 8:377–380; 1983.
6. De Vries, G. J.; Buijs, R. M. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Res 273:307–317; 1983.
7. De Vries, G. J.; Buijs, R. M.; Van Leeuwen, F. W.; Caffe, A. R.; Swaab, D. F. The vasopressinergic innervation of the brain in normal and castrated rats. J. Comp. Neurol. 233:236–254; 1985.
8. De Vries, G. J.; Duetz, W.; Buijs, R. M.; Van Heerikhuize, J.; Vreeburg, J. T. M. Effects of androgens and estrogens on the vasopressin and oxytocin innervation of the adult rat brain. Brain Res. 399:296–302; 1986.
9. Garner, D.; McGivern, R. F.; Jaegels, G.; Laks, M. M. A new chronic method for direct measurement of systolic and diastolic pressures in the conscious rat: Vascular access ports. Lab. Anim. Sci. 38:205–207; 1988.
10. Gallaher, E. J.; Egner, D. A. Rebound hyperthermia follows ethanol-induced hypothermia in rats. Psychopharmacology (Berl.) 91:34–39; 1987.
11. Giri, P. R.; Dave, J. R.; Tabakoff, B.; Hoffman, P. L. Arginine vasopressin induces the expression of c-fos in the mouse septum and hippocampus. Mol. Brain Res. 7:131–137; 1990.
12. Gulya, K.; Dave, J. R.: Hoffman, P. L. Chronic ethanol ingestion decreases vasopressin mRNA in hypothalamic and extrahypothalamic nuclei of mouse brain. Brain Res. 557:129–135; 1991.
13. Hoffman, P. L. Central nervous system effects of neurohypophyseal peptides. In: Smith, C. W., ed. The peptides. New York: Academic Press; 1987:239–295.
14. Hoffman, P. L.; Ritzmann, R. F.; Walter, R.; Tabakoff, B. Arginine vasopressin maintains ethanol tolerance. Nature 276: 614–616; 1978.
15. Israel, Y.; Khanna, J. M.; Orrego, H.; Rachamin, G.; Wahid, S.; Britton, R.; MacDonald, A.; Kalant, H. Studies on the metabolic tolerance to alcohol, hepatomegaly and alcoholic liver disease. Drug Alcohol Depend. 4:109–129; 1979.
16. Kasting, N. W. Criteria for establishing a physiological role for brain peptides. A case in point: The role of vasopressin in thermo-

regulation during fever and antipyresis. Brain Res. Rev. 14:143–153; 1989.
17. Le, A. D.; Kalant, H.; Khanna, J. M. Interaction between des-9-glycinamide-[8-Arg]vasopressin and serotonin on ethanol tolerance. Eur. J. Pharmacol. 80:337–345; 1982.
18. Linkola, J.; Fyhrquist, F.; Forsander, O. Effects of ethanol on urinary arginine vasopressin excretion in two rat strains selected for their different ethanol preferences. Acta Physiol. Scand. 101: 126–128; 1977.
19. Mannix, S. A; Hoffman, P. L.; Melchior, C. L. Intraventricular arginine vasopressin blocks the acquisition of ethanol tolerance in mice. Eur. J. Pharmacol. 128:137–141; 1986.
20. Melchior, C. L.; Hoffman, P. L.; Tabakoff, B. Influencing environment-dependent tolerance to ethanol. In: Cicero, T. J., ed. NIAAA research monograph 13. Washington, DC: U.S. Government Printing Office; 1983:242–292.
21. Mezey, E.; Potter, J. J. Effect of castration on the turnover of rat liver alcohol dehydrogenase. Biochem. Pharmacol. 34:369–372; 1985.
22. Peris, J.; Cunningham, C. L. Handling-induced enhancement of alcohol's acute physiological effects. Life Sci. 38:273–279; 1986.
23. Peris, J.; Cunningham, C. L. Stress enhances the development of tolerance to the hypothermic effect of ethanol. Alcohol Drug Res. 7:187–193; 1987.
24. Pittman, Q. J.; Malkinson, T. J.; Kasting, N. W.; Veale, W. L. Enhanced fever following castration: Possible involvement of brain arginine vasopressin. Am. J. Physiol. 254 (Reg. Integr. Comp. Physiol. 23):R513–R517; 1988.
25. Pittman, Q. J.; Rogers, J.; Bloom, F. E. Arginine vasopressin deficient Brattleboro rats fail to develop tolerance to the hypothermic effects of ethanol. Reg. Peptides 4:33–41; 1982.
26. Siegel, S. Pharmacological conditioning and drug effects. In: Goudie, A. J.; Emmett-Oglesby, M. W., eds. Psychoactive drugs. Clifton, NJ: Humana Press; 1989:115–180.
27. Szabo, G.; Tabakoff, B.; Hoffman, P. L. Receptors with V_1 characteristics mediate the maintenance of ethanol tolerance by vasopressin. J. Pharmacol. Exp. Ther. 247:536–541; 1988.
28. Tribollet, E.; Audigier, S.; Dubois-Dauphin, M.; Dreifuss, J. J. Gonadal steroids regulate oxytocin receptors but not vasopressin receptors in the brain of male and female rats. An autoradiographical study. Brain Res. 511:129–140; 1990.