Pharmacology Biochemistry & Behavior, Vol. 21, pp. 539–543, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Neurohypophyseal Peptides Maintain Tolerance to the Incoordinating Effects of Ethanol

PAULA L. HOFFMAN*[1] AND BORIS TABAKOFF*†

**Alcohol and Drug Abuse Research and Training Program, Department of Physiology and Biophysics*
University of Illinois at Chicago, Health Sciences Center
and †Westside VA Medical Center, Chicago, IL 60612

Received 3 February 1984

HOFFMAN, P. L. AND B. TABAKOFF. *Neurohypophyseal peptides maintain tolerance to the incoordinating effects of ethanol.* PHARMACOL BIOCHEM BEHAV 21(4) 539–543, 1984.—Arginine vasopressin (AVP), lysine vasopressin (LVP) and [des-9-glycinamide]LVP (DGLVP), administered systemically, delayed the disappearance of functional tolerance to the motor-incoordinating effect of ethanol in mice. This result is consistent with previous findings that AVP and related neuropeptides maintain tolerance to the sedative-hypnotic and hypothermic effects of ethanol, and suggests that the peptides modulate the rate of disappearance of tolerance *per se*, rather than simply influencing the tests used to evaluate tolerance. However, both the duration of tolerance to the incoordinating effect of ethanol, and the duration of peptide maintenance of this tolerance, were less than those observed for tolerance to the hypnotic and hypothermic effects of ethanol. Tolerance to various effects of ethanol clearly can develop and dissipate at different rates, and our results suggest that the characteristics of the maintenance of ethanol tolerance by neurohypophyseal peptides are influenced, to some extent, by the neural systems which mediate the expression of the functional tolerance which is being investigated.

Alcohol Arginine vasopressin Ataxia Ethanol tolerance Lysine vasopressin Mice Neurohypophyseal peptides

TOLERANCE to ethanol, as to other drugs, is defined as a decreased response of an animal or human to a given dose of ethanol, following previous exposure to ethanol [19]. Ethanol tolerance has recently been recognized to be a complex phenomenon, the expression of which is influenced by a number of physiological and environmental factors [19]. In particular, functional tolerance to various behavioral and physiological effects of ethanol develops at different rates [18, 19, 21], and the development and maintenance of tolerance to each effect may involve changes in the function of specific neurochemical systems.

It has previously been demonstrated that the neurohypophyseal hormone, arginine vasopressin (AVP), as well as structurally related peptides, can maintain functional tolerance to the sedative-hypnotic and hypothermic effects of ethanol in mice [2,6]. These peptides appeared to modulate tolerance *per se*, and not simply to influence the physiological responses used to evaluate tolerance [2,5]. To further assess the generality of maintenance of ethanol tolerance by neurohypophyseal peptides, we have, in the present study, evaluated the ability of AVP and other peptides to maintain tolerance to a different effect of ethanol, namely ethanol-induced incoordination, or ataxia, in mice.

METHOD

All peptides, with the exception of DGAVP and LVP, were synthesized in the laboratory of Dr. R. Walter, Department of Physiology and Biophysics, University of Illinois Medical Center, and were from the same batches used in earlier studies [2,6,24]. The LVP used was from the laboratory of Dr. V. du Vigneaud, and had been isolated from porcine pituitary. [Des-9-glycinamide]AVP (DGAVP) was supplied by Organon, Oss, Holland (Batch TN-738-AK_1). AVP had approximately 400 U/mg of rat pressor activity, and LVP had about 240 U/mg of rat pressor activity [12].

Male C57Bl/6J mice (23–25 g) were used in all experiments. Mice were housed six per cage under conditions of controlled temperature (22±1°C) and lighting (12-hour light/dark cycle) for at least one week prior to being used in an experiment. Ethanol was administered by a liquid diet technique used previously in our laboratories [15]. Briefly, mice were individually housed and acclimated for one day to a liquid diet containing Carnation Slender, vitamin supplement (3 g/l; ICN Corp., Cleveland, OH), and sucrose (96.8 g/l) (control diet). For the next seven days, control mice continued to receive the same diet, while ethanol-treated mice

[1]Requests for reprints should be addressed to Paula L. Hoffman, PhD, Department of Physiology and Biophysics, University of Illinois at Chicago (HSC), P.O. Box 6998, Chicago, IL 60680.

were given a diet in which ethanol (7% v/v) equicalorically replaced the sucrose. The amount of diet offered to the control animals was adjusted daily to match the average amount consumed by the ethanol-consuming mice. On the morning of the eighth day, all mice were again given control diet (i.e., the ethanol diet was withdrawn from ethanol-consuming mice). This regimen produced functional ethanol tolerance and physical dependence (defined by the appearance of previously-described withdrawal symptomatology [15]) in the ethanol-treated mice. Withdrawal symptoms were monitored at two-hour intervals for 12 hours following withdrawal [15]. At 24 hours after withdrawal, when overt withdrawal symptoms had dissipated, mice were tested for tolerance to the incoordinating effects of ethanol, using an accelerating Rotarod Treadmill for mice (Ugo Basile Co.) [8]. To assess ethanol-induced motor incoordination, animals were placed on the Rotarod for five minutes of acclimation with the Rotarod moving at 3 rpm, and in the absence of acceleration. If, on any particular day, a mouse was unable to maintain itself on the Rotarod during this period, it was not utilized for that day's experiment. Acceleration was then begun (the apparatus accelerates from 3 to 30 rpm over a period of five minutes), and the time at which each animal fell from the Rotarod was noted. If the animal did not fall, it was removed from the apparatus after 800 seconds. The result (i.e, time to fall) of this first test of the animals on the Rotarod will be referred to throughout as the "pre-ethanol performance." Following this test, animals were injected IP with ethanol. In preliminary studies, a dose-response curve for the effect of ethanol was determined using doses of 1.0 to 2.5 g/kg of ethanol, and a dose giving approximately 70–80% impairment in control animals (2.3 g/kg; 14.9% v/v solution) was chosen for tolerance testing (see Table 1). Twenty minutes after the ethanol injection, animals were again placed on the rotating (but non-accelerating) Rotarod for one minute. Acceleration was initiated, and the time at which each animal fell was noted (maximum, 800 seconds). The result of this second test of the animals on the Rotarod will be referred to as the "post-ethanol performance." The difference between each animal's pre- and post-ethanol performances was calculated as:

$$\text{Percent Impairment} = \frac{\text{pre-ethanol performance} - \text{post-ethanol performance}}{\text{pre-ethanol performance}} \times 100$$

so that each animal served as its own control. Any *increase* in time on the Rotarod during the post-ethanol test was socred as 0% impairment.

Tolerance testing took place at 9:00 a.m. and, following the initial tolerance test (at 24 hours after withdrawal), groups of control (C) and ethanol-withdrawn (E) mice were subdivided into groups which received, at 4:00–6:00 p.m., a subcutaneous injection of peptide (C-AVP, C-DGAVP, C-LVP, C-DGLVP, C-Oxytocin; and corresponding E-peptide groups) or saline vehicle (C-Sal, E-Sal) [2, 5, 6]. Peptides were dissolved in saline immediately prior to use, and were administered at a dose of 400 nmole/kg body weight. Mice continued to receive SC injections of peptide or saline once daily in the evening for seven days, and were tested for tolerance on the mornings (9:00 a.m.) of days two through five and day 8 after withdrawal.

A given experiment included two to three groups of ethanol-treated mice and two to three groups of control mice (i.e., a saline-treated group and either a group treated with one peptide, or two groups treated with different peptides). In general, there were ten to twelve ethanol-treated mice per group, and six to ten control mice per group. Three or four experiments were carried out using each peptide, with the exception of DGLVP (one experiment). Data from E-Sal and C-Sal groups were pooled over all experiments, and data for each peptide group were pooled over all experiments using that peptide. Therefore, each C-peptide group (except C-DGLVP) represents about 30 animals. The number of animals in each E-peptide group is indicated in the legend to Fig. 1. Statistical analysis of results was accomplished by the use of analysis of variance (using the arcsin transformation where appropriate) and the Tukey-Kramer test [16], or by Student's t-test. A value of $p<0.05$ was considered as significant.

TABLE 1

DOSE-DEPENDENCE OF ETHANOL-INDUCED MOTOR INCOORDINATION MEASURED ON THE ACCELERATING ROTAROD TREADMILL FOR MICE

Ethanol Dose (g/kg)	Percent Impairment
0	11 ± 6 (17)
1.0	14 ± 9 (8)
1.5	24 ± 9 (8)
2.0	60 ± 12 (22)*
2.5	86 ± 6 (9)*
AVP + 1.5	35 ± 8 (10)†

C57Bl mice were tested on the accelerating Rotarod prior to and 20 minutes after an IP injection of the indicated dose of ethanol. Percent impairment refers to the difference in pre- and post-ethanol performance of each animal; improved performance after ethanol injection was rated as 0% impairment (for details, see text). AVP (400 nmole/kg, SC) was administered 16 hours prior to testing wth 1.5 g/kg ethanol ("AVP+1.5"). Values represent mean ± SEM; numbers in parentheses are numbers of animals.

*$p<0.01$ compared to saline-treated mice (t-test).

†Not significantly different from mice treated with 1.5 g/kg of ethanol alone; $p<0.05$ compared to saline-treated mice (t-test).

RESULTS

The mice showed a wide range of abilities to remain on the Rotarod during the pre-ethanol performance. However, the percent impairment produced by ethanol was less variable, and this impairment was dose-dependent in ethanol-naive animals (Table 1). A dose of 2.3 g/kg of ethanol was expected, from these preliminary results, to induce approximately 70–80% impairment in control mice, and was used to assess the development and maintenance of tolerance to ethanol. The administration of AVP (400 nmole/kg, SC) to untreated mice 16 hours prior to testing on the Rotarod (the interval used in the chronic experiments) did not significantly affect the response to ethanol (Table 1).

Ingestion of ethanol in a liquid diet for seven days resulted in tolerance to the motor-incoordinating effect of ethanol, when mice were tested at 24 hours after withdrawal (Fig. 1). The dose of 2.3 g/kg of ethanol caused 25.7±2.3% impairment (mean±SEM; n=154) in the ethanol-treated animals, compared to 87.9±1.3% impairment in the control animals (n=79; $p<0.001$ compared to ethanol-withdrawn;

t-test). The impairment caused by ethanol in the liquid diet-fed control mice was similar to what had been observed in ethanol-naive mice (Table 1). In each chronic experiment, control and ethanol-treated mice were tested once daily for five days after withdrawal. The pre-ethanol performance of the C-Sal group (n=34) improved over time, so that by five days after withdrawal, many animals remained on the Rotarod for the full 800 seconds prior to ethanol injection. Treatment of control animals with AVP (n=34) did not alter the rate of improvement in the pre-ethanol performance (ANOVA; effect of days, $F(4,322)=13.36, p<0.001$; effect of treatment (saline or AVP), $F(1,322)=0.59$, NS; day × treatment interaction, $F(4,322)=0.52$, NS). Although the pre-ethanol performance of control mice improved over days, the percent impairment produced by ethanol in the C-Sal group did not change significantly over days (Fig. 1 and see below). Similarly, daily treatment of control animals (n=20–40 per group) with AVP, DGAVP, [8-lysine]vasopressin (LVP) or oxytocin did not significantly affect the response to ethanol on any day (ANOVA; effect of days, $F(4,688)=1.20$, $p<0.31$; effect of treatment, $F(4,688)=2.35$, $p>0.05$, day × treatment interaction, $F(16,688)=0.54$, NS). While the effect of treatment appeared to be marginally significant on the basis of the ANOVA, analysis by the Tukey-Kramer test revealed that the response to ethanol of each of the C-peptide groups was not significantly different from that of the C-Sal group on any day. There was insufficient DGLVP available to treat control animals for longer than two days. However, there was no significant difference in the response of the C-DGLVP group compared to that of the C-Sal group on day 2 or 3 after withdrawal (Student's *t*-test).

The *pre*-ethanol performance on the Rotarod of the chronically ethanol-treated mice did not differ significantly from that of control mice on any day (ANOVA, effect of treatment [E-Sal vs. C-Sal] $F(1,275)=1.44$; NS), and improved similarly to the performance of control mice with daily testing. However, in contrast to the control mice, percent impairment caused by ethanol in these animals also changed over time after withdrawal, as they lost tolerance to the motor-incoordinating effect of ethanol. Thus, mice in the E-Sal group became continuously more sensitive to the motor-impairing effect of ethanol over five days after withdrawal (Fig. 1). By the fourth day after withdrawal, the response of these animals was no longer significantly different from that of controls (Fig. 1; see statistical analysis in legend). In contrast, animals in the E-AVP group remained tolerant to the motor-incoordinating effect of ethanol (i.e., the response of ethanol-withdrawn, AVP-treated animals *was* significantly different from that of control animals) on the fourth and fifth days after withdrawal. The response of the mice in the E-AVP group returned to the level of that of control mice by eight days after withdrawal (Fig. 1). Very similar results were obtained with animals in the E-LVP or E-DGLVP groups (Fig. 1). On the other hand, ethanol-withdrawn mice treated with oxytocin or with DGAVP lost tolerance at a rate similar to that seen in the E-Sal group: i.e., oxytocin and DGAVP did not maintain tolerance to the motor-incoordinating effect of ethanol.

Since DGAVP had previously been found to maintain tolerance to the sedative-hypnotic and hypothermic effects of ethanol in mice [2,14], we assessed the chemical purity of the batch of DGAVP used in the present study. Thin-layer chromatography on silica gel in two solvent systems ($BuOH/HOAc/H_2O$[4:1:1]; $BuOH/HOAc/H_2O/Pyr$[15:3;12:

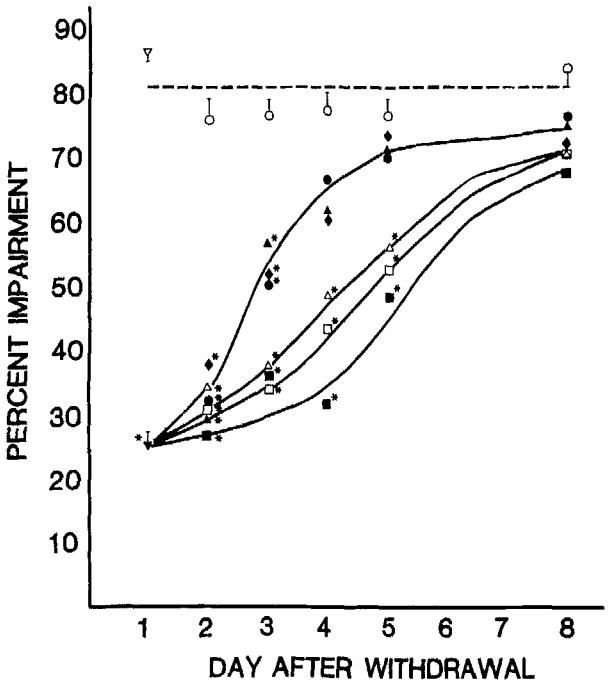

FIG. 1. Effect of neurohypophyseal peptides on dissipation of tolerance to the motor-incoordinating effect of ethanol. Mice were fed a liquid diet containing ethanol or an equicaloric amount of sucrose (controls) for seven days. Tolerance to ethanol was determined by measuring the difference in amount of time the animals could remain on an accelerating Rotarod treadmill before and after an injection (IP) of 2.3 g/kg of ethanol (percent impairment produced by ethanol). Animals received the indicated peptide (400 nmole/kg) or saline once daily, beginning on the day after withdrawal, as described in the text. Values represent the mean±SEM of percent impairment produced by ethanol in mice in the C-Sal group (○), and the mean percent impairment (SEM left out for clarity) of mice in the E-Sal (●) (n=70–79), E-AVP (△) (n=45–51), E-DGAVP (▲) (n=35–38), E-LVP (□) (n=33). E-DGLVP (■) (n=11) or E-Oxytocin (◆) (n=21–24) groups. Percent impairment on the first day after withdrawal (▽ control; ethanol-treated ▼) was determined prior to peptide treatment. Ethanol-induced impairment in C-peptide animals did not differ significantly from that in C-Sal animals at any time (see text), and the data represents reponses of C-Sal animals (n=79). The response of these control animals to ethanol did not vary significantly over days (see text). All groups of ethanol-treated animals gradually lost tolerance to ethanol, but at different rates (ANOVA; effect of days, $F(4,1330)=57.64$, $p<0.001$; effect of treatment, $F(6,1330)=31.04, p<0.001$; treatment × day interaction, $F(24,1330)=845.31$, $p<0.001$). Analysis by the Tukey-Kramer test showed that all groups of ethanol-withdrawn animals remained tolerant on the second and third days after withdrawal (*$p<0.05$, compared to controls). On the fourth and fifth days after withdrawal, animals in the E-AVP, E-LVP and E-DGLVP groups were still tolerant (*$p<0.05$, compared to controls), while the response of animals in the E-Sal, E-oxytocin and E-DGAVP groups was no longer significantly different from that of controls. A single line on the graph is used to represent the response of the latter three groups. On the eighth day after withdrawal, there were no significant differences in response among the groups.

10]), with detection by the chlorine-tolidine method [13], revealed a single component. Amino acid analysis (ratios: Cys, 2.58; Tyr, 0.82; Phe, 0.99; Glx, 1.00; Asx, 0.98; Pro, 0.91; Arg, 0.96) indicated that the peptide was 91.5% pure.

DISCUSSION

AVP, as well as LVP and DGLVP, delayed the loss of tolerance to the motor-incoordinating effect of ethanol. This finding is consistent with previous observations that arginine vasopressin and structurally related peptides can maintain tolerance to the sedative-hypnotic and hypothermic effects of ethanol in mice, as well as to the hypothermic and motor-impairing effects of ethanol in rats [2, 6, 9, 14]. The results support the hypothesis that the peptides modulate a mechanism underlying the maintenance of ethanol tolerance *per se*, rather than affecting the particular behaviors that are used to assess tolerance.

The liquid diet technique used in the present study induces functional ethanol tolerance in the mice, and does not produce metabolic tolerance [15]. In addition, we have previously shown that vasopressin treatment does not alter the rate of ethanol metabolism during tolerance testing [4]. Therefore, the tolerance maintained by peptide treatment can be defined as functional tolerance. The characteristics of peptide maintenance of tolerance to the incoordinating effects of ethanol in mice are similar in many respects to those of peptide maintenance of tolerance to the hypnotic and hypothermic effects of ethanol [2, 6, 7]. Oxytocin is inactive in maintaining tolerance to all of the tested effects of ethanol in mice, while LVP and AVP are approximately equipotent [2,6]. Previous dose-response and structure-activity studies indicated that these differential responses to various peptides were not simply a result of differences in peptide distribution or metabolism after systemic injection, although such factors could play a role, but most likely reflected interactions with specific CNS peptide receptors [2,4]. In addition, the fact that DGLVP, a synthetic peptide which was shown to be essentially devoid of peripheral endocrinological activity [24], can maintain tolerance to the motor-incoordinating effects of ethanol as well as to the other effects of ethanol [2], is in line with a central site of action for the peptides in modulating tolerance.

There are, however, some differences in the influence of the peptides on tolerance to various effects of ethanol. One major difference is the time course of peptide action. In our previous studies, AVP maintained tolerance to the hypnotic and hypothermic effects of ethanol at a constant level for as long as it was administered, up to 15 days after withdrawal [2,6]. However, tolerance to the motor-incoordinating effect of ethanol in AVP-, LVP- and DGLVP-treated mice was gradually lost during the five-day testing period, and even when peptide administration was continued up to the seventh day after withdrawal, the animals were no longer tolerant on the eighth day. The time course for dissipation of tolerance to the incoordinating effects of ethanol in saline-treated mice (four to five days; Fig. 1) is also somewhat shorter than that for disappearance of tolerance to the sedative-hypnotic or hypothermic effects of ethanol (six to nine days), when tolerance is produced by the identical liquid diet protocol [2,15]. It is possible that the duration of peptide action in maintaining tolerance to a particular effect of ethanol is related to the rate of dissipation of that particular tolerance. Crabbe and Rigter, for example, found that treatment of mice with DGAVP enhanced tolerance to the hypothermic effect of ethanol at one day after removal of mice from an ethanol inhalation chamber, but not at two days after withdrawal, [1,14]. In their studies, tolerance had already dissipated in the vehicle-treated mice after one day of withdrawal [1].

Another difference between the present study and our previous work is the ineffectiveness of DGAVP in maintaining tolerance to the incoordinating effect of ethanol. DGAVP was previously found to be quite potent in maintaining tolerance to the hypnotic effect of ethanol [2], and it is therefore unlikely that differences in uptake, metabolism or distribution of DGAVP, in comparison to the active peptides, account for its ineffectiveness in the present study. The lack of response to DGAVP in the present experiments is also not simply due to the decreased peripheral endocrinological activities of DGAVP, since DGLVP, which is also devoid of these activities, was equally as active as AVP or LVP in maintaining tolerance. For similar reasons, it seems unlikely that the lack of the C-terminal glycinamide residue *per se* led to the lack of potency of DGAVP, although it is possible that there are very stringent structural requirements for maintenance of the particular tolerance measured in this study. While the DGAVP appeared to be relatively pure, as assessed both by amino acid analysis and by thin-layer chromatography, it is quite difficult to accurately determine the purity of such small peptides except by bioassay. Due to the lack of peripheral activity of DGAVP, this was not possible. We have previously found that different batches of neurohypophyseal peptides can vary considerably in potency [2], and one must be cautious in the interpretation of negative results under these circumstances.

It has previously been demonstrated, using a moving-belt apparatus which also measures motor incoordination, that ethanol tolerance can develop more rapidly in animals which are repeatedly tested under the influence of ethanol, compared to animals which are tested prior to receiving ethanol [10]. This phenomenon has been called "behaviorally augmented" tolerance [10]. Although, in our experiments, animals did improve with practice in their pre-ethanol performance on the Rotarod, the control animals, whether saline- or peptide-treated, did not develop tolerance to the effect of ethanol during the five days of testing. Thus, behaviorally augmented tolerance was not evident in our testing paradigm, and peptide treatment did not enhance the development of behaviorally-augmented tolerance. The peptides appeared only to delay the loss of tolerance which was already present in the ethanol-treated animals. It is of interest to note that AVP treatment did not affect the rate of improvement in the pre-ethanol performance of control animals on the Rotarod. Thus, this aspect of "learning" was not modified by vasopressin, although there have been some reports that neurohypophyseal peptides can affect acquisition, as well as retrieval, of learned responses [e.g., 11, 22, 23, 24].

In summary, our results are compatible with the hypothesis that AVP and related peptides may modulate the activity of neurochemical systems which are involved in the maintenance of tolerance to many of the physiological and behavioral effects of ethanol. We have previously discussed, with respect to ethanol tolerance [20], a concept which also applies to neurobiologic studies of memory, i.e., that of intrinsic and extrinsic neural systems which participate in consolidation and storage of memory [17]. This concept may provide a framework for understanding vasopressin's effects on tolerance. Intrinsic systems are those which encode spe-

cific information, e.g., tolerance to a particular effect of ethanol, presumably by alterations in synaptic efficacy. Extrinisic systems are those which can influence the development, maintenance or expression of tolerance, but do not encode tolerance in themselves [17,20]. Our previous studies suggested a role for central noradrenergic systems both in the development of functional tolerance to the hypnotic and hypothermic effects of ethanol [20], and in the maintenance of tolerance to these effects by vasopressin in mice [3]. Thus, noradrenergic systems may be regarded as extrinsic systems which modulate tolerance, and which are, in turn, modulated by vasopressin. However, differences in the neuronal systems which directly influence the behavioral or physiological functions which become tolerant to ethanol—i.e., intrinsic systems—may contribute to differences in the duration of functional tolerance, and in the length of time for which neurohypophyseal peptides can prolong the expression of tolerance.

ACKNOWLEDGEMENTS

The authors thank Dr. T. S. Raju for excellent technical assistance. We are grateful to Organon, Oss, Holland for the generous gift of DGAVP. This work was supported in part by grants from ADAMHA, USPHS (AA-3817; AA-5372; AA-2696), the VA Medical Research Service, and the Banbury Foundation. P.L.H. is the recipient of an ADAMHA Research Scientist Development Award (AA-52). B.T. is the recipient of an ADAMHA Research Scientist Award (AA-63).

REFERENCES

1. Crabbe, J. C., Jr. and H. Rigter. Hormones, peptides and ethanol responses. In: *Alcohol Tolerance and Dependence*, edited by J. C. Crabbe, Jr. and H. Rigter. Amsterdam: Elsevier/North Holland: Biomedical Press, 1980, pp. 293–316.
2. Hoffman, P. L. Structural requirements for neurohypophyseal peptide maintenance of ethanol tolerance. *Pharmacol Biochem Behav* **17:** 685–690, 1982.
3. Hoffman, P. L., C. L. Melchior and B. Tabakoff. Vasopressin maintenance of ethanol tolerance requires intact brain noradrenergic systems. *Life Sci* **32:** 1065–1071, 1983.
4. Hoffman, P. L., R. F. Ritzmann and B. Tabakoff. The influence of arginine vasopressin and oxytocin on ethanol dependence and tolerance. In: *Currents in Alcoholism*, vol 5, edited by M. Galanter. New York: Grune and Stratton, 1979, pp. 5–16.
5. Hoffman, P. L., R. F. Ritzmann and B. Tabakoff. Neurohypophyseal peptide influences on ethanol tolerance and acute effects of ethanol. *Pharmacol Biochem Behav* **13:** Suppl 1, 279–284, 1980.
6. Hoffman, P. L., R. F. Ritzmann, R. Walter and B. Tabakoff. Arginine vasopressin maintains ethanol tolerance. *Nature* **276:** 614–616, 1978.
7. Hoffman, P. L. and B. Tabakoff. Centrally-acting peptides and tolerance to ethanol. In: *Currents in Alcoholism*, vol 8, edited by M. Galanter, New York: Grune and Stratton, 1981, pp. 359–378.
8. Jones, B. J. and D. J. Roberts. The quantitative measurement of motor incoordination in naive mice using an accelerating Rotarod. *J Pharm Pharmacol* **20:** 302–304, 1968.
9. Le, A. D., H. Kalant and J. M. Khanna. Interaction between des-9-glycinamide-[8-Arg]vasopressin and serotonin on ethanol tolerance. *Eur J Pharmacol* **80:** 337–345, 1982.
10. LeBlanc, A. E., R. J. Gibbins and H. Kalant. Behavioral augmentation of tolerance to ethanol in rat. *Psychopharamcologia* **30:** 117–122, 1973.
11. Messing, R. B. and S. B. Sparber. Des-gly-vasopressin improves acquisition and slows extinction of autoshaped behavior. *Eur J Pharmacol* **89:** 43–51, 1983.
12. *Pharmacopeia of the United States, 18th Revision.* Easton: Mack Publishing Company, 1970, p. 771.
13. Randerath, K. *Thin-Layer Chromatography*. New York: Academic Press, 1966, p. 123.
14. Rigter, H., H. Rijk and J. C. Crabbe. Tolerance to ethanol and severity of withdrawal in mice are enhanced by a vasopressin fragment. *Eur J Pharmacol* **64:** 53–68, 1980.
15. Ritzmann, R. F. and B. Tabakoff. Body temperature in mice: A quantitative measure of alcohol tolerance and physical dependence. *J Pharmacol Exp Ther* **199:** 158–170, 1976.
16. Sokal, R. R. and F. J. Rohlf. *Biometry, 2nd Edition*. San Francisco: W. H. Freeman and Co., 1981, p. 242ff.
17. Squire, L. R. and H. P. Davis. The pharmacology of memory: A neurobiological perspective. *Annu Rev Pharmacol Toxicol* **21:** 323–256, 1981.
18. Tabakoff, B. and K. Kiianmaa. Does tolerance develop to the activating, as well as the depressant, effects of ethanol? *Pharmacol Biochem Behav* **17:** 1073–1076, 1982.
19. Tabakoff, B., C. L. Melchior and P. L. Hoffman. Commentary on ethanol tolerance. *Alcoholism: Clin Exp Res* **6:** 252–259, 1982.
20. Tabakoff, B. and R. F. Ritzmann. The effects of 6-hydroxydopamine on tolerance to and dependence on ethanol. *J Pharmacol Exp Ther* **203:** 319–332, 1977.
21. Tabakoff, B., R. F. Ritzmann, T. S. Raju and R. A. Deitrich. Characterization of acute and chronic tolerance in mice selected for inherent differences in sensitivity to ethanol. *Alcoholism: Clin Exp Res* **4:** 70–73, 1980.
22. van Ree, J. M., B. Bohus, D. H. G. Versteeg and D. de Wied. Neurohypophyseal principles and memory processes. *Biochem Pharmacol* **27:** 1793–1800, 1978.
23. Vawter, M. P. and K. F. Green. Effects of desglycinamide-lysine vasopressin on a conditioned taste aversion in rats. *Physiol Behav* **25:** 851–854, 1980.
24. Walter, R., P. L. Hoffman, J. B. Flexner and L. B. Flexner. Neurohypophyseal hormones, analogues and fragments: Their effect on puromycin-induced amnesia. *Proc Natl Acad Sci USA* **72:** 4180–4184, 1975.