Pharmacology Biochemistry & Behavior, Vol. 10, pp. 229–234. Printed in the U.S.A.

Effects of Desglycinamide(9)-Lysine(8)-Vasopressin and Prolyl-Leucyl-Glycinamide on Oral Ethanol Intake in the Rat

R. F. MUCHA
AND
H. KALANT

Addiction Research Foundation of Ontario
33 Russell Street, Toronto, Canada M5S 2S1
and
Department of Pharmacology, University of Toronto
Toronto, Canada M5S 1A8

(Received 18 September 1978)

MUCHA, R. F. AND H. KALANT. *Effects of desglycinamide[9]-lysine[8]-vasopressin and prolyl-leucyl-glycinamide on oral ethanol intake in the rat.* PHARMAC. BIOCHEM. BEHAV. **10**(2) 229–234, 1979.—Rats given ethanol in their drinking water at a concentration that permitted adequate fluid intake gradually accepted higher concentrations and consumed larger amounts of ethanol. These increases were augmented when daily subcutaneous injections of 1 μg of desglycinamide[9]-lysine[8]-vasopressin (DGLVP) or 10 μg of prolyl-leucyl-glycinamide (PLG) were given concomitantly. Nonsignificant changes in ethanol consumption were seen with injections of 1 μg PLG, or 0.42 or 42 μg of lysine[8]-vasopressin (LVP). In a second experiment 4 μg DGLVP given every second day as a long-acting zinc phosphate complex, commencing after the increases in ethanol intake had taken place, failed to produce any change in ethanol consumption subsequently. In both Experiments 1 and 2, the rats were switched from forced ethanol intake to a choice between ethanol and tap water. On these tests there was only marginal evidence of peptide-produced changes in ethanol intake.

Ethanol Forced ethanol intake Voluntary ethanol intake Pituitary peptides
Desglycinamide[9]-lysine[8]-vasopressin Prolyl-leucyl-glycinamide Lysine[8]-vasopressin

IT WAS demonstrated previously that desglycinamide[9]-lysine[8]-vasopressin (DGLVP), an octapeptide prepared by cleaving the terminal glycine from lysine[8]-vasopressin (LVP), influenced oral ethanol consumption in intact rats [7]. Naive rats, forced to consume low but gradually increasing concentrations of ethanol in their drinking water, and injected subcutaneously with microgram quantities of DGLVP, eventually accepted higher concentrations and consumed larger amounts of ethanol than rats injected with vehicle containing no DGLVP. The DGLVP-treated rats also showed higher voluntary consumption of ethanol than the vehicle control group during a subsequent period of free choice between the previously accepted ethanol concentration and plain tap water.

The purpose of the present experiments was to replicate and extend these findings in at least two different ways. First, DGLVP is only one of a number of peptides, related to the hormones of the posterior pituitary, which have been reported to have similar behavioral effects. LVP, for example, produces effects resembling those of DGLVP on various models of learning [1, 6, 8]. In addition, prolyl-leucyl-glycinamide (PLG), the tripeptide sidechain of oxytocin, has been reported to produce a facilitation of opiate tolerance and physical dependence analogous to that of DGLVP [19]. It was of interest, therefore, to determine whether LVP, DGLVP, and PLG would also have similar influences on the development of ethanol drinking behavior. Second, it was desirable to determine whether there was a critical period for the peptide effect. In the earlier study [7], DGLVP was given throughout the acquisition period and resulted in different levels of ethanol intake. Therefore, in the second experiment to be described here, the peptide was given for a limited time after the subjects had been brought to a stable level of forced ethanol intake.

METHOD

Animals

The animals were male Sprague-Dawley rats purchased from Canadian Breeding Laboratories (Constant, Quebec). They had initial body weights of 325–400 g in Experiment 1, and 250–350 g in Experiment 2. They were maintained in single cages with ad lib access to Purina Laboratory Chow, in a room at 20°C with lights on at 0800 and off at 2000 hr. Each rat had access to two Richter-type drinking tubes throughout its stay in the colony; the solutions in the tubes varied according to the stage of the experiment. Initially, the rats received tap water in both tubes.

Drug Preparations

Ethanol solutions were prepared from tap water and 95% ethanol. Concentrations were expressed as percent of total volume constituted by the volume of 95% ethanol. Solutions were made 1–7 days before use, and were stored at 4°C.

All the peptides were kindly donated by Organon (Oss, The Netherlands) through the courtesy of Drs. E. Schönbaum and H. Rigter. The LVP had a potency of 242 international pressor units (U) per mg, as assayed by Organon. All peptide solutions were prepared every second day, stored at 4°C until use, and administered in a volume of 0.2 ml. In Experiment 1, the peptides were dissolved in physiological saline. In Experiment 2, the peptide was given as a zinc-phosphate suspension. This vehicle permits a sustained release of peptide for up to 48 hr [5].

Procedure

Experiment 1. The general design of Experiment 1 was very similar to that of the earlier report [7]. Seventy-two rats were studied during a 46-day period in which individual fluid consumption was monitored daily at 1000 to 1300 hr. Every second day, the rats were weighed and the drinking bottles were filled with fresh solutions. The experiment had three consecutive phases differing only in the presence or absence of peptide injections, and in the nature of the drinking fluids presented.

The first phase of the experiment consisted of a 6-day period in which the baseline water consumption was measured. These data were used to divide the rats into six groups, and derive an estimate of each rat's normal volume of fluid consumption for a 2-day period. The second phase lasted 26 days and involved two aspects. First, the solution in both drinking tubes was ethanol. The starting ethanol concentration was 1% and it was varied individually for each rat through a series of concentration steps consisting of 3, 5, 9, 11, 13, 15 and 18%. Decisions to change the concentration were made following each 2-day period and were based on the drinking behavior of individual animals. Whenever the volume of fluid consumed over the 2-day period exceeded 82% of the rat's baseline intake, the ethanol concentration for the next 2-day period was increased by one step. However, if less than 82% was consumed various changes in concentration were possible. The concentration was kept the same if it had been changed during the previous 4 days. If the concentration was the same over the previous two consecutive 2-day periods and still less than 82% of baseline fluid intake was consumed, the concentration was dropped one step. It was dropped by two steps if less than 75% of the baseline fluid volume had been consumed. Throughout the second phase, the six groups were also given vehicle, 1 μg DGLVP, 1 μg PLG, 10 μg PLG, 42 μg LVP (10 U), or 0.42 μg LVP (0.1 U), respectively. Injections were given subcutaneously (SC) at the back of the neck between 1600 and 1700 hr, commencing on the last baseline day and ending 26 days later. The third phase of the experiment involved offering a choice between ethanol in one drinking tube and tap water in the other. The ethanol concentration for an individual rat consisted of the last concentration that the rat consumed in a volume greater than 82% of the baseline water intake. Each rat was offered this concentration for 8 days and was offered a concentration one step lower for 6 more days. Bias in the data due to side preference was minimized by randomly selecting the relative positions of the ethanol and water bottles every 2 days.

Experiment 2. The procedure was very similar to that of Experiment 1 except that DGLVP, in a zinc-phosphate buffer, was administered only once every second day in a dose of 4 μg per rat, and the starting ethanol concentration was 3% instead of 1%.

Forty-three rats were started on the 6-day baseline water consumption phase, then were placed on the regimen of forced ethanol consumption for 14 days. Concentrations of ethanol were determined individually as in Experiment 1. On Day 15, the animals were divided into two groups balanced on the basis of daily ethanol consumption. That afternoon, 21 rats were injected with DGLVP and 22 with vehicle. Each rat received four more similar injections at 2-day intervals. After the last injection, all rats were offered a choice of water and ethanol solution over the next 12 days.

Data Analyses

The data were analyzed by the methods of Kirk [9]. *A priori* hypotheses were advanced for alcohol intake and alcohol acceptance concentrations on the basis of the previous study [7]. Overall analyses of variance were used to determine the error terms. Dunn's procedure was used to evaluate individual mean comparisons and the experimentwise error was set at $p<0.05$. Alcohol intake data were plotted as mean g/kg of ethanol consumed during each two-day block. This served largely to eliminate a sawtooth appearance caused by a consistently decreased level of consumption on the first day of each block. This decrease was not relevant to the present experiments since the group differences in drinking patterns were always present on both days of each block. In addition, the analyses of variance were carried out before averaging over the two-day blocks. Since no a priori hypotheses were advanced for changes in body weight, the data were first analyzed by an overall analysis of variance followed by Tukey HSD tests for individual mean comparisons. The actual analyses were carried out on the weight gains above the last baseline weight; gains were computed by subtracting the weight of the last baseline day from those of each of the 20 two-way blocks. This was necessary since the mean weights of the rats at the start of the experiment were not quite equal.

RESULTS

Experiment 1. The results of the experiment are summarized in the first three figures. The mean daily alcohol intakes (Fig. 1) confirmed the previous finding [7] that DGLVP increased the forced ethanol intake, and showed

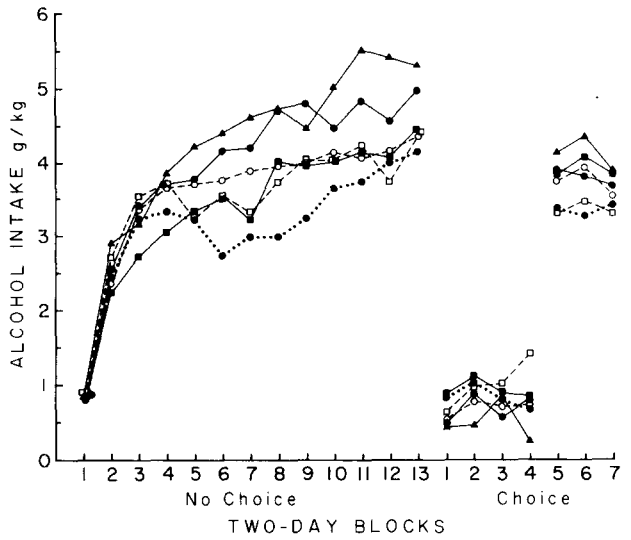

FIG. 1. Mean ethanol consumption during forced and choice drinking periods, for groups injected daily for 26 days with saline (●....), 1 μg DGLVP (▲——), 1 μg PLG (○– – –), 10 μg PLG (●——), 42 μg LVP (■——), or 0.42 μg LVP (□– – –). On Day 5 of the choice the ethanol concentration was lowered one concentration.

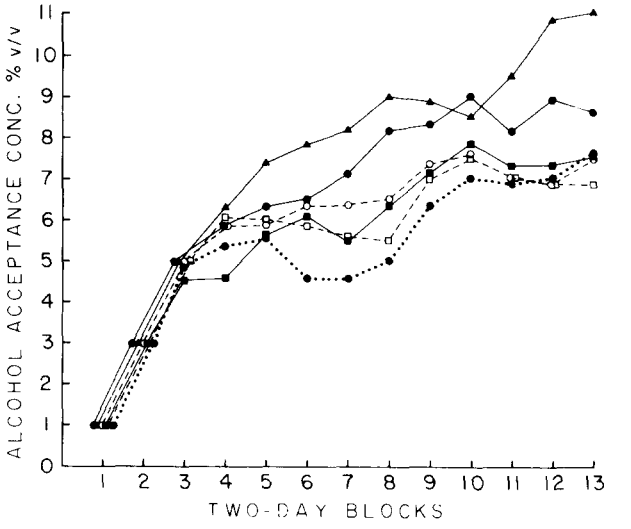

FIG. 2. Mean concentration of ethanol consumed during forced ethanol consumption for the groups in Fig. 1.

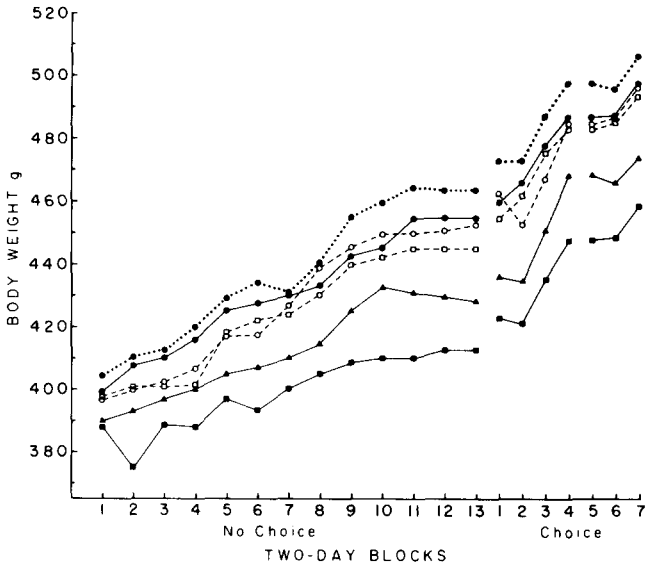

FIG. 3. Mean body weight of groups in Figs. 1 and 2 during Experiment 1.

that PLG had a similar effect. Over the first three two-day blocks, every rat in each group more than doubled its daily ethanol intake. Thus, on the third block, with the exception of a tendency in the 42 μg LVP group towards a lower level of intake relative to the remaining five groups, $F(1,1716)=2.36$, $p>0.05/6$, all treatment groups exhibited a similar level of ethanol intake. However, between Block 3 and Block 13, there was no appreciable increase in the amount consumed by the control, whereas there was for the DGLVP and 10 μg PLG groups; the difference between the means of Block 3 and 13 for the DGLVP, $F(1,1650)=9.17$, $p<0.05/3$, was significant. The 42 μg LVP group also showed a similar increase over the period, $F(1,1650)=7.5$, $p<0.05/3$; however, this may have been partly due to the lower level consumed on the third block. Comparisons of the group means over the final 10 blocks indicated that the DGLVP, $F(1,1716)=13.3$, $p<0.05/6$, and the 10 μg PLG, $F(1,1716)=9.95$, $p<0.05/6$, group consumed significantly more than the saline-treated rats. Similar comparisons of the control to the 42 μg LVP group failed to indicate significance; however, both 1 μg PLG, $F(1,1716)=5.48$, $p>0.05/6$, and 0.42 μg LVP, $F(1,1716)=4.42$, $p>0.05/6$, showed strong tendencies towards significance. Since the total fluid intake for each rat was kept fairly constant during the forced ethanol consumption phase by varying the ethanol concentrations, the same pattern of results was observed when the concentrations of ethanol consumed over two-day blocks were plotted (Fig. 2). Over the final 10 two-day blocks the saline-treated rats were significantly different from the DGLVP, $F(1,858)=10.28$, $p<0.05/3$, and the 10 μg PLG group, $F(1,858)=6.39$, $p<0.05/3$, but not from any other groups. The 1 μg PLG group did not even approach a level significantly different from the control, $F(1,858)=2.26$, $p>0.05/3$.

During the first 8 days of the ethanol choice period (Fig. 1) there were no differences between the groups, but when the ethanol concentration was lowered one step there was a tendency for the peptide groups to drink higher doses of ethanol. However, statistical tests failed to find the means of any individual peptide-treated group significantly different from those of the control.

The mean body weights of the rats in the present experiment are shown in Fig. 3. Analyses of the amount of weight gained during the course of the experiment indicated a clear group-by-block interaction, $F(5,1254)=1.63$, $p<0.05$. Comparisons of the combined data from the last four blocks of the forced ethanol consumption period indicated that the saline group gained significantly more weight than the LVP (Tukey's $q=5.97$, $p<0.05$). There was also a tendency for the

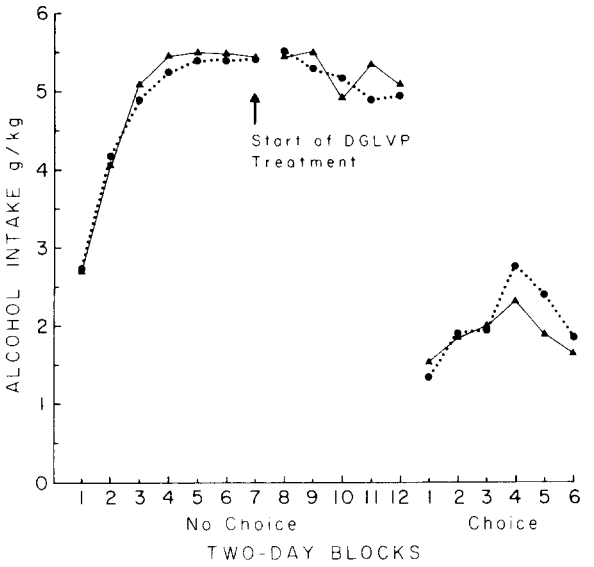

FIG. 4. Mean ethanol consumption during forced and choice drinking periods for groups injected every second day for 10 days, commencing on Day 14, with zinc phosphate vehicle (●....) or vehicle plus 4 μg DGLVP (▲——).

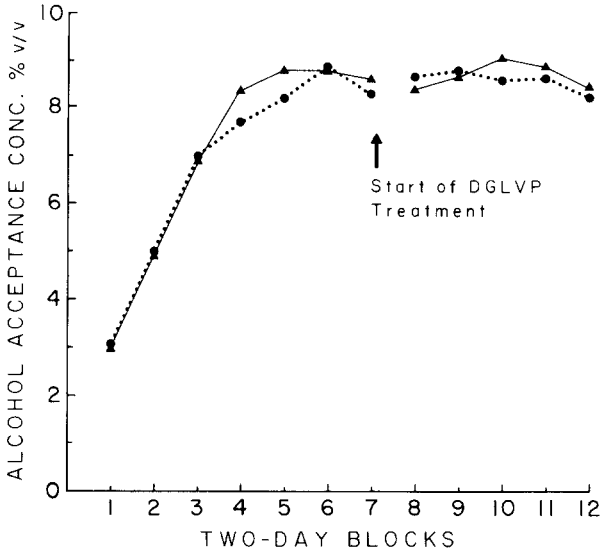

FIG. 5. Mean concentration of ethanol consumed during forced ethanol consumption for groups in Fig. 4.

DGLVP group to be low relative to the saline, but it was not significant (Tukey's $q=3.59$, $p>0.05$, critical $q=4.00$).

Experiment 2. The data are presented in Figs. 4 and 5. It is clear that neither the alcohol intakes (Fig. 4) nor the alcohol acceptance concentrations (Fig. 5) were affected by DGLVP administration. Similarly, during the choice ethanol drinking period (see Fig. 4) there were no significant differences between the two treatment groups. The body weights of these two groups did not differ at any point after the presentation of DGLVP.

DISCUSSION

Experiment 1 indicated that daily administration of 1 μg DGLVP or 10 μg PLG can augment the increase in amount and concentration of ethanol consumed during forced ethanol consumption. These results replicated our previous finding [7] and indicated that peptide-produced changes in ethanol-intake behavior were not specific to DGLVP. Although there were tendencies in the data the most surprising finding was that LVP did not produce a similar augmentation even though the two employed doses of LVP were equivalent to or even higher than the doses reported to have effects on conditioned avoidance [6]. It is possible that the 0.42 μg of LVP was, however, too low for this particular experimental paradigm. In addition, the influence of LVP on body-weight gain (Fig. 3) argued that the 42 μg LVP rats were not comparable to those in the other groups. The mechanism of this effect is not clear; however, it is probably not due to the water retaining property of LVP since that should have caused an increase in body weight [3]. There may have been an endocrine central effect, since there was also a tendency for DGLVP to produce a weight suppression.

The results of Experiment 2 clearly indicated that the administration of DGLVP following the increase in ethanol consumption produced almost no change in subsequent ethanol drinking behavior. The absence of a peptide effect could not be attributed to methodological problems since the group sizes were large. In addition, the DGLVP effects were assessed against stable baselines of ethanol drinking behavior. Such baselines have in the past been very effective in detecting changes in ethanol drinking produced by other agents (cf. [14]). Furthermore, it is unlikely that stress of injections masked an effect. Five injections were given and the animals were handled every second day for their weighing; this allowed ample time for habituation to take place. Also, these injections rarely caused any reaction in the animals. Finally, the failure to see effects cannot be due to the dose of DGLVP or the vehicle of administration since Finkelberg *et al.* [7] used this regimen and mode of peptide administration and found effects with doses that were equal to or one quarter the size of the present. It was concluded that DGLVP likely alters processes that occur only during the initial period of forced ethanol exposure.

It should be noted that the control group in Experiment 2 attained a much higher acceptance level and daily intake than the vehicle controls in Experiment 1. This does not seem to be related to the use of a zinc phosphate vehicle in Experiment 2, since the injections were not started until the animals had reached a stable intake. The difference is consistent with our previous experience that different batches of animals, even from the same supplier, vary substantially in alcohol consumption. The level of intake by the controls in Experiment 2 was very similar to that seen by Finkelberg *et al.* [7].

The results of the present experiments also tended to suggest that the higher level of choice ethanol consumption in DGLVP-treated rats seen previously [7] and to a slight degree in Experiment 1 was a minor effect, likely related to the increased drinking during the forced ethanol period. First, the DGLVP-related increase in consumption of ethanol during the choice period in Experiment 1 was of marginal magnitude relative to the effects seen during the forced

period. We can not yet explain the difference in findings during the choice period in Experiment 1 and in the earlier study. Second, administration of DGLVP for 10 days prior to the choice period in Experiment 2 failed to cause any increase in the levels of ethanol intake during the choice period and this was concomitant with a failure to see increased intake during the last part of the forced period.

A number of psychological factors are involved in determining the level of ethanol intake by rats (cf. [12,13]) and it is possible that DGLVP and PLG may influence any one of them. Although some of these factors may be ruled out, precisely how the peptides altered ethanol intake must remain speculative. Our data suggested that the peptides influence processes occurring during the period of initial exposure to the ethanol. Thus, factors which are thought to determine ethanol intake at any point of ethanol exposure (e.g., taste threshold, motivation for water) are likely not involved. There are a number of factors that may be involved during the early exposure to ethanol. First, these peptides may alter a process of habituation to the aversive effects of the alcohol [13]. Our model does not allow us to determine whether ethanol intake under these conditions is determined primarily by the motivating effects of ethanol or by the aversiveness of its taste. Second, a learning process may take place during the development of the drinking behavior, and the peptides may influence processes of consolidation and memory, as has been suggested for other behaviors [10,16]. Third, DGLVP may subtly influence positive reinforcement [18] and, if ethanol intake goes up because of the positively reinforcing properties [12], alterations of these will change the level eventually consumed. Failure to see effects in Experiment 2 may be related to the fact that in many instances an established behavior is more resistant to changes of reinforcement than those undergoing acquisition [4]. Fourth, there is a punishment process in our paradigm that may be attenuated in the peptide-treated animals: on each 2-day period that a rat consumes a quantity of ethanol greater than 82% of its baseline it is punished for doing so by getting a more aversive concentration of ethanol to consume.

The literature on the psychological effects of the presently employed peptides also fails to provide a clear locus for the peptide effect on ethanol intake. On the basis of various reports, one may postulate that the present effects were due to a specific alteration of motivation [18], consolidation [10], retrieval [16] or attention [17]. However, such postulates may not be fruitful since they are spawned by data suggesting that the effects of the peptides vary with the behaviors under study. Van Ree and De Wied [18], for example, found that DGLVP decreased and PLG increased the acquisition of a barpressing response reinforced by intravenously administered heroin. The acquistion and retention of a pole-jumping response to avoid footshock was increased by DGLVP [6, 11] and the retention of an acquired avoidance of a flavored solution paired with lithium chloride-induced illness was, in some instances, inhibited by PLG and not changed in others [15]. Moreover, both DGLVP and PLG potentiated the acquisition of morphine tolerance and dependence [19] and protected mice from amnesia for a learned response produced by puromycin [10,20]. Thus, a unitary postulate cannot be derived to account for the present and previously reported PLG- and DGLVP-produced effects unless more is known about the relative contribution of the aforementioned psychological processes in each of the behaviors and how the peptides interact with these processes.

The similar effect of both DGLVP and PLG on ethanol intake is consistent with the results found in studies of morphine tolerance and dependence [19] and puromycin-induced amnesia [20]. It has been suggested that C-terminal neurohypophyseal peptides may comprise the structural requirements for activity in the present paradigm. However, we cannot rule out the possibility that DGLVP and PLG produced their effects by activating different receptors. Additional peptides must be tested for effects on ethanol intake. Pentobarbital-induced sleeping time was altered by a wide range of natural and synthetic peptides that lack any apparent common structure and endocrine relation [2]. It is apparent, therefore, that considerable information is required before the effects of peptides on ethanol intake can be satisfactorily understood.

ACKNOWLEDGEMENTS

The assistance of L. Currin, T. Fahlgren, K. Potter, C. Brenner and K. Voshart is gratefully acknowledged.

REFERENCES

1. Ader, R. and D. De Wied. Effects of lysine vasopressin on passive avoidance learning. *Psychon. Sci.* **29:** 46–48, 1972.
2. Bissette, G., C. B. Nemeroff, P. T. Loosen, G. F. Breese, G. B. Burnett, M. B. Lipton and A. J. Prange, Jr. Modification of pentobarbital-induced sedation by natural and synthetic peptides. *Neuropharmacology* **17:** 229–237, 1978.
3. Chan, W. Y. A study on the mechanism of vasopressin escape: effects of chronic vasopressin and overhydration on renal tissue osmolality and electrolytes in dogs. *J. Pharmac. exp. Ther.* **184:** 244–252, 1973.
4. Deese, J. and S. H. Hulse. *The Psychology of Learning*. New York: McGraw-Hill, 1967, p. 113.
5. De Wied, D. Inhibitory effects of ACTH and related peptides on extinction of conditioned avoidance behaviour in rats. *Proc. Soc. exp. Biol. Med.* **122:** 28–32, 1966.
6. De Wied, D., H. M. Greven, S. Lande and A. Witter. Dissociation of the behavioural and endocrine effects of lysine vasopressin by tryptic digestion. *Br. J. Pharmac.* **45:** 118–122, 1972.
7. Finkelberg, F., H. Kalant and A. E. LeBlanc. Effect of vasopressin-like peptides on consumption of ethanol by the rat. *Pharmac. Biochem. Behav.* **9:** 453–458, 1978.
8. Hostetter, G., S. L. Jubb and G. P. Kozlowski. Vasopressin affects the behavior of rats in a positively-rewarded discrimination task. *Life Sci.* **218:** 1323–1328, 1977.
9. Kirk, P. E. *Experimental Design Procedures for the Behavioral Sciences*. Belmont, CA: Brooks/Cole, 1968.
10. Lande, S., J. B. Flexner and L. B. Flexner. Effect of corticotropin and desglycinamide^{9}-lysine vasopressin on suppression of memory by puromycin. *Proc. natn. Acad. Sci. U.S.A.* **69:** 558–560, 1972.
11. Lande, S., A. Witter and D. de Wied. An octapeptide that stimulates conditioned avoidance acquisition in hypophysectomized rats. *J. Biol. Chem.* **246:** 2058–2062, 1971.
12. Mello, N. K. A review of methods to induce alcohol addiction in animals. *Pharmac. Biochem. Behav.* **1:** 90–101, 1973.
13. Myers, R. D. and W. L. Veale. The determinants of alcohol preference in animals. In: *The Biology of Alcoholism, Vol. II: Physiology and Behavior*, edited by H. Begleiter and B. Kissin. New York: Plenum Press, 1972, pp. 131–167.
14. Pinel, J. P. J. and R. F. Mucha. Suppression of voluntary ethanol consumption in rats by electroconvulsive shock. *Physiol. Behav.* **15:** 585–591, 1975.

15. Rigter, H. and A. Popping. Hormonal influences on the extinction of conditioned taste aversion. *Psychopharmacologia* **46:** 255–261, 1976.
16. Rigter, H., H. Van Riezen and D. De Wied. The effects of ACTH- and vasopressin-analogues on CO_2-induced retrograde amnesia in rats. *Physiol. Behav.* **13:** 381–388, 1974.
17. Sandman, C. A. and W. D. Alexander. Neuroendocrine influences on visual discrimination and reversal learning in the albino and hooded rat. *Physiol. Behav.* **11:** 613–617, 1973.
18. Van Ree, J. M. and D. De Wied. Modulations of heroin self-administration by neurohypophyseal principles. *Eur. J. Pharmac.* **43:** 199–202, 1977.
19. Van Ree, J. M. and D. De Wied. Prolyl-leucyl-glycinamide (PLG) facilitates morphine dependence. *Life Sci.* **19:** 1331–1340, 1976.
20. Walter, R., P. L. Hoffman, J. B. Flexner and L. B. Flexner. Neurohypophyseal hormones, analogs, and fragments: their effect on puromycin-induced amnesia. *Proc. natn. Acad. Sci. U.S.A.* **72:** 4180–4184, 1975.