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Effect of Vasopressin-Like Peptides on Consumption of Ethanol by the Rat

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FINKELBERG, F., H. KALANT AND A. E. LE BLANC. *Effect of vasopressin-like peptides on consumption of ethanol by the rat.* PHARMAC. BIOCHEM. BEHAV. 9(4) 453–458, 1978.—Rats were trained to accept ethanol in their drinking water, by successive small increments or decrements in alcohol concentration in response to the individual consumption of each rat. Those injected with desglycinamide^9-lysine^8-vasopressin (DGLVP), 1–4 μg SC every second day, attained almost twice as high a final acceptance concentration (FAC) and mean daily ethanol intake (g/kg) as vehicle-treated controls. Hypophysectomized animals initially accepted the same alcohol concentrations as intact rats, but drank much larger volumes and correspondingly higher daily g/kg intakes. However, this was rapidly succeeded by rejection of all but very low concentrations, which was unaffected by DGLVP. During a subsequent free-choice period (water vs. ethanol at individual FACs), the groups maintained their relative positions with respect to ethanol intake. This was not altered by injection of vasopressin in the hypophysectomized rats, but was overcome by raising the alcohol concentration. The results suggest that vasopressin-like peptides facilitate acquisition of alcohol drinking behavior.

Vasopressin DGLVP Alcohol consumption Rat Hypophysectomy Alcohol preference Aversion Taste threshold

DESGLYCINAMIDE^9-lysine^8-vasopressin (DGLVP), produced from lysine vasopressin (LVP) by cleavage of the N-terminal glycine residue of the sidechain, has no antidiuretic hormone activity but has a prolonged effect against the extinction of conditioned avoidance behavior [6,31]. Hypophysectomy and hereditary diabetes insipidus severely impair the retention of the same behavior [6,8]. An effect similar to that of DGLVP has been demonstrated with a number of pituitary peptide hormones, chiefly adrenocorticotrophin (ACTH), LVP and fragments of these peptides, given during or at the end of the training period [6,7]. The effect is unexplained, but has been reproduced repeatedly, and almost certainly results from a direct action of these substances on specific central nervous system receptors. Minute biochemical manipulation of the ACTH molecule, or of ACTH fragments without adrenocortical effect, can decrease or even reverse the expected maintenance of conditioned behavior [16]. Similar manipulations of the molecule of LVP have been carried out [7,10].

There has been very little investigation of the effects of DGLVP on the acquisition or retention of appetitive behaviors, though other pituitary peptides have been reported to improve acquisition of lever pressing for water [17] or heroin [30] reward, and maze learning for water [31], food [6, 22, 29, 31] and sexual reinforcement [1]. It would be of considerable theoretical and practical interest to explore the effect of DGLVP on a different type of appetitive behavior, the oral consumption of novel substances such as drug solutions.

DGLVP is found in pituitary fractions isolated from normal rats [24]. It is not known whether synthesis or secretion of DGLVP is affected by drugs. However, endogenous release of the closely related LVP, as well as of ACTH, occurs in response to a variety of stimuli including barbiturates and chlorpromazine [15], morphine [14], nicotine [4], and probably amphetamines [26] and tetrahydrocannabinol [23]. In contrast, ethanol inhibits the release of vasopressin [11], which is the most potent of the behaviorally active peptides and which potentiates the release of ACTH [34]. Since ethanol also inhibits release of oxytocin [13], and does not stimulate release of ACTH except at moderately high blood alcohol levels [20,28], it is reasonable to expect that it would not cause release of endogenous DGLVP as other drugs might. Thus, any effect of exogenous DGLVP might be easier to interpret in an alcohol study than in the case of other drugs.

The present report deals with an investigation of the effect of DGLVP on the acquisition and retention of alcohol-drinking behavior (ADB). The study used a new training procedure, BARTENDER [12], which increases the ethanol concentration very gradually and according to the individual response elicited. Parallel groups of hypophysectomized animals were also run, to indicate whether loss of endogenous pituitary peptides would influence acquisition of ethanol consumption, and whether exogenous peptides would restore the normal pattern.

GENERAL METHOD

Ninety-day old Sprague-Dawley albino rats (Charles River, Inc., Cambridge, Mass.) were housed singly, with lab

TABLE 1

DESIGN OF EXPERIMENT. SEE TEXT FOR EXPLANATION OF SYMBOLS

Time	Procedure
Surgical preparation	Hypophysectomy or sham operation 14 days recovery
Pretest 6 days	Injection and tube practice 4-day water/water baseline. Begin substitution therapy in hypox.
Training 24 days	Alcohol/alcohol forced-choice
Test 24 days	Alcohol/water free choice employing alcohol at the predetermined FAC. Further manipulation of hormone treatment (see text).

Operation Condition	Dose level	Sequence of treatments	n
Hypox	low	XX	(18*)
		TX	12
		TT	12
	medium	XX	18*
		TX	24
		TT	24
	high	XX	(18*)
		TX	12
		TT	12
Sham	low	XX	(18†)
		TX	12
		TT	12
	medium	XX	18†, R(30)
		TX	18
		TT	18
	high	XX	(18†)
		TX	12
		TT	12

Note: Data for low- and high-dose vehicle-treated groups were duplicated from the middle-dose condition: (18*) from 18*, (18†) from 18†. 'R(30)' indicates a replication of the sham-operated placebo-treated (medium-dose) group; see text for details.

chow and drinking fluid available ad lib throughout the experiments. Two Richter drinking tubes were provided for each cage; in the following description, the contents of the two bottles are specified (e.g., water/water) for each portion of the experiment. Fluid consumption from each bottle was recorded daily.

Baseline water intake (water/water) was recorded for four days and then alcohol acceptance was monitored over a full 24-day training period [12]. Alcohol was given in both tubes (alcohol/alcohol) and, provided the individual animal drank a volume equal to at least 80% of baseline fluid intake, the concentration was raised for that animal every two days from 1% v/v to 3, 5, 7, 9, 11, 13, 15, 18, 21, 25 and 30% v/v. Rejection (defined as intake of less than 80% of baseline) led to temporary leveling or even lowering of concentration in the step sequence, until a final acceptance concentration or FAC was determined for each animal at the end of training. This FAC was then offered throughout a 24-day free-choice (alcohol/water) test.

Chemical Preparation

DGLVP was prepared according to the method of De Wied *et al.* [9], by tryptic digestion of pure porcine LVP (batch KS 538B) kindly donated by Organon, Inc., Oss, The Netherlands. A slow-release, long-acting zinc phosphate-hydroxide complex of DGLVP was prepared from dry peptide every 8 days to ensure potency [5]. Vehicle placebo was made up in exactly the same way as the peptide solution, to compensate for a reported slight stimulatory effect of zinc salts [25].

Placebo vehicle injections were begun, where appropriate, one day prior to the beginning of the baseline fluid recording; peptide injections, where appropriate, replaced the vehicle on the day prior to the beginning of the alcohol/alcohol training. Injections were given SC every 48 hr in all groups.

Data Analysis

The data from each experiment, as well as the data for each measure, were treated by balanced analysis of variance [33], performed by the Fortran program BALANOVA (Dr. Paul Herzberg, York University, Toronto).

Experimental Design

Ninety-day-old male Sprague-Dawley albino rats were hypophysectomized (hypox; n = 114) or sham-hypophysectomized (sham; n = 94) via the pharyngeal route by the supplier. The completeness of hypophysectomy was verified by autopsy at the end of the experiment, as well as by baseline water intakes. The animals were assembled into 14 treatment groups (Table 1). Only the intermediate dose of placebo was used but, to accommodate the requirements of a balanced analysis of variance, synthetic data for low- and high-dose placebo groups in each operation condition were derived by duplicating data from the intermediate dose group, with an appropriate reduction in degrees of freedom.

The design represents a full cross of four factors (with the exception just described): operation (hypox, sham); dose level of DGLVP (low = 1 μg, medium = 2 μg, high = 4 μg); sequence of treatments (XX = placebo only, i.e., peptide never given; TX = peptide given during training and placebo during test; TT = peptide given during both training and test); and time (in days). The 2 μg dose in these animals was comparable to the 3.5 μg/kg dose which Lande *et al.* [24] had found to be highly effective in maintaining avoidance behavior.

All hypox animals received replacement therapy SC, consisting of desoxycorticosterone acetate (DOCA: Ciba Percorten, 0.50 mg) in sesame oil, plus sodium levothyroxine (T4: Baxter, 25 μg) and dexamethasone sodium phosphate (Decadron: Merck, Sharp and Dohme, 8 μg) in physiological saline. These were given every 48 hr throughout the experiment, beginning on the eighth day preceding training.

Groups XX represent a control condition. A comparison of TX and TT groups was expected to show whether continuation of peptide treatment into the test period was necessary to maintain any observed increase in alcohol intake.

Test Period

Days 1–8, A/W choice. Each animal was offered a choice

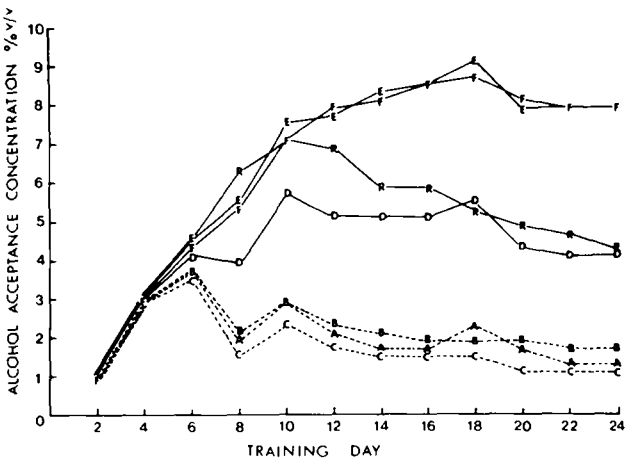

FIG. 1. Daily mean acceptance concentrations of ethanol during the alcohol training period. Solid lines indicate sham-operated groups, and broken lines indicated hypophysectomized groups. A, D and R received placebo injections only; B and E received DGLVP injections throughout the training period only; C and F received DGLVP throughout both the training and the subsequent alcohol/water test period.

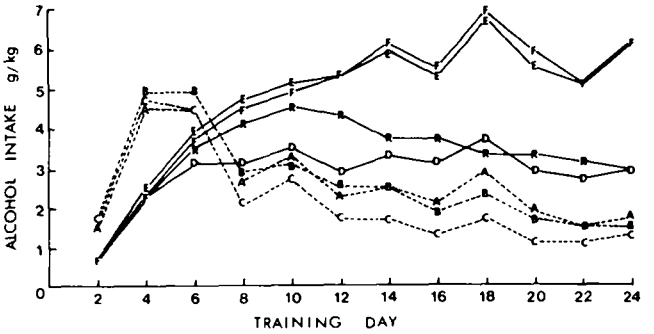

FIG. 2. Daily mean ethanol consumption during the alcohol training period. Lines and symbols as explained in legend to Fig. 1 and in Table 1.

of water and alcohol solution at its individual FAC. The relative positions of the two Richter tubes were varied according to a computer-generated semirandom schedule which limited the repetition of recognizable position sequences.

Days 9–16, LVP manipulation of dehydration factor. The low g/kg alcohol consumption by hypox animals (see Results) might have been due to dehydration, since these animals lack LVP. If so, alcohol intake might be increased by correcting water need. Beginning on Test Day 9, hypox TX and TT animals were therefore given LVP (Sandoz AG, Basel; synthetic, batch 71005) and DGLVP was discontinued, while the XX group continued on placebo. Sham animals were divided the same way. The LVP doses were 0.50 (low), 1.0 (medium) or 2.0 I.U. (high), all given SC every 48 hr.

Days 17–24, manipulation of dilution factor in hypox animals. The procedures of Days 9–16 were continued, but the ethanol concentration for each animal was raised by two steps in the sequence (usually 4% more). This was done because nearly all hypox animals had ended training at a FAC of only 1% v/v, which would require them to drink very great volumes of fluid to achieve more than minimal alcohol consumption (g/kg).

Replication (Group R)

A replication of the sham-placebo group (n=30) was subsequently run through the same procedures up to the end of the training period, R(30) in Table 1. Additional analyses of variance, as well as post hoc comparisons tested by the Scheffé procedure [33], were conducted to compare Group R with the three other sham groups.

RESULTS

Training Period

Hypox animals, as expected, had a higher baseline water intake than sham animals (240 vs. 110 ml/kg/day; $F(1,202)=278$, $p<0.001$). The water baselines of all sham groups, including Group R, were comparable.

Training

The *daily acceptance concentrations* (DACs) for ethanol during training are shown in Fig. 1. As noted below, effects of the 3 doses of DGLVP did not differ significantly from each other. Therefore the results for the three dosage subgroups within each treatment group are pooled in the figures. The DACs initially rose in very smooth fashion in the various treatment groups. However, while the DACs of sham animals continued to rise, there was a major early decline in the acceptance concentrations of all hypox animals after only six days of training. $F(23,4646)=108$, $p<0.001$. After Day 6, the mean DAC for hypox animals was 1.0% v/v and for sham animals, 6.9%.

There was also a marked divergence of mean DAC during training for the three sham groups, $F(46,4646)=6.0$, $p<0.001$. The steepest rises occurred before Day 10. After this, placebo-treated sham animals reached a plateau (Days 10–18), then exhibited a slow decline. DGLVP-treated sham animals continued to accept higher concentrations until Day 18.

On Day 25, 94% of hypox animals ended at FAC's of 1% v/v, while 68% of sham animals were at FACs of 7% or higher, $F(1,202)=210$, $p<0.001$. Among sham animals, DGLVP produced a significantly greater proportion of high FACs than placebo did, $F(2,202)=8.7$, $p<0.001$.

The same general relationships appeared when training-period alcohol intake (g/kg) was examined (Fig. 2). Hypox and sham animals differed both in peak intake and in their behavior after peak intake, $F(23,4646)=105$, $p<0.001$. Early during training, the hypox animals were drinking at concentrations similar to those of the sham animals but took in much larger volumes owing to water loss, and therefore had a higher g/kg intake. However, by the fourth day, their intake stopped increasing, then fell asymptotically to a level well below that of the sham animals.

Among sham animals, intake rose smoothly in the second week, and subsequently showed no major decline. DGLVP-treated sham animals drank steadily more than their placebo controls after the first week, rising to 6 g/kg/day with no subsequent decline. In contrast, average intake in the placebo-sham group dropped about 12% over the last week, $F(46,4646)=6.27$, $p<0.001$.

The dose × operation × peptide effect was nonsignificant in this analysis, as were all other dose effects, including

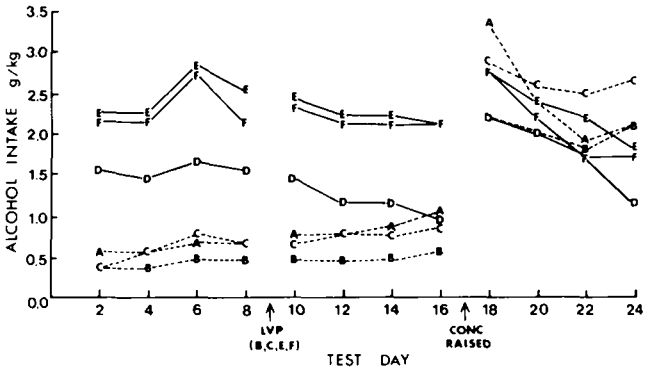

FIG. 3. Daily mean ethanol consumption during the free-choice (alcohol/water) test period. Lines and symbols as explained in legend to Fig. 1. From Day 9 on, Groups B, C, E and F were given synthetic LVP SC every two days, in doses of 0.5–2.0 I.U. From Day 17 on, the concentration of ethanol solution offered to all groups was raised above each animal's FAC by approximately 4% (v/v). See text for details of these changes.

dose×peptide. This assured that the major operation×peptide effect displayed in Fig. 2 was not due to the use of a single placebo dose condition and the subsequent handling of data.

Group R reached FACs nearly identical with those in the original placebo/sham group (n=18). Group R had a higher peak acceptance concentration than the original group on days 7–12 of the training period, $F[3(F_{.999}(3,2944))]=92.1$, $p<0.001$, and a higher peak intake, $F[3(F_{.999}(3,2944))]=56.7$, $p<0.001$, but these were still markedly lower than in the peptide/sham groups run earlier, $F[3(F_{.999}(3,2944))]=51.8$, $p<0.001$; $F=136.5$, $p<0.001$.

Test Period

Despite a general decline in intake in all groups to about half the respective previous levels as soon as the alcohol/water choice test began, the overall pattern seen during the training continued for the first two weeks (Fig. 3). Because of the procedural differences in successive eight-day blocks, separate analyses were carried out for the whole test period and for the first 16 days.

In the 16-day analysis of alcohol intake, the operation×peptide×days interaction was nonsignificant, indicating that the separation of the curves shown in Fig. 2 was maintained throughout these first 16 days (operation×peptide differences, $F(2,202)=5.32$, $p<0.005$). The modest decline in intake over Days 6–16 of test, for example, was parallel in placebo-treated and peptide-treated sham animals. Thus, the pattern of treatment effects previously established in sham animals was undisturbed by the shift in peptide treatment after Day 8.

Sham animals did drink more alcohol than hypox animals over the first 16 days, $F(1,202)=73.0$, $p<0.001$, but the slowly declining intake in sham groups and slight rise in intake in hypox groups between Day 10 and Day 16 together produced some convergence, as shown by a significant days×operation interaction, $F(15,3030)=6.93$, $p<0.001$.

Because of the low FACs of alcohol to which they were exposed during the test, the hypox animals could have drunk large amounts of alcohol (g/kg) prior to Day 17 only by increasing their total fluid intake drastically. But they did not do so, and volume intake of fluids actually declined even further in each of the hypox groups after LVP treatment began on Day 9, from about 70% of baseline to about 60% in the TX and to 55% in the TT group. However, all of the reduction in fluid intake occurred in the water portion; hypox animals continued to drink at least as much alcohol as before.

LVP did not decrease the overall fluid intake of sham animals below the stable levels reached at the end of training and during the first week of test. These remained close to or above baseline in all sham groups. When ethanol concentration was raised by two steps above the individual FACs (Days 17–24), the differences between hypox and sham groups and between DGLVP and placebo groups disappeared completely (Fig. 3).

DISCUSSION

Sham-Operated Animals

DGLVP produced a more lasting pattern of alcohol drinking behavior (ADB) in essentially normal animals, when given during early drug experience. This effect resembles those of several pituitary peptides in maintaining appetitive behaviors during extinction, as noted in the introduction.

The effects of the three different doses of DGLVP used here did not differ. This suggests that all doses were supramaximal, and that dose-response relationships remain to be defined. Also, it did not matter whether peptide treatment was extended into the test period or not.

The replicated placebo-sham Group R exhibited more pronounced peak DAC's and a higher peak intake than the original placebo-sham Group D, but its sequential behavior was otherwise closely comparable. There was a rapid increase in alcohol acceptance (acquisition of ADB), followed by gradual extinction, but acceptance always exceeded the initial baseline. However, these differences between the two placebo groups suggest two possible mechanisms of peptide action. Group R reached a prominent peak of intake and then declined gradually, while Group D stabilized at a lower plateau from which there was no apparent decline. The first suggests that the peptide facilitates the acquisition of drinking behavior, while the second suggests that it prevents extinction. This issue is the subject of experiments to be reported separately.

Hypophysectomized Animals

The unusual pattern of consumption by the hypox animals during the training period presumably reflects the balance of two or more physiological processes. The need for fluid replacement led to much higher volume intake than by the controls during the first 4 days. However, this also involved a much higher ethanol intake in g/kg, which appears to have invoked an active aversion to ethanol and a consequent fall in intake. This is consistent with Crow's observations in rats with median eminence lesions [3]. The early strong alcohol rejection by hypox animals arises from some consequence of hypophysectomy not reversed by DGLVP. One may wonder which hormone, among those not given in substitution therapy, is responsible for ordinary levels of acceptability of alcohol.

Finally, the long-maintained rejection of ethanol by the hypox animals (DACs usually 1%) indicates that alcohol is more aversive to hypox than to normal animals. The increased aversiveness might be due to changes in metabolism,

changes in central nervous system sensitivity, altered taste sensitivity or other mechanisms. For example, it is known that corticosteroid deficiency is associated with increased sensory acuity in both laboratory animals and humans [18]. Hypox animals may therefore have greater taste sensitivity to alcohol as well as to other substances. However, this explanation would be difficult to reconcile with two additional pieces of evidence. The first is the initial high intake of alcohol by the hypox animals during the training period. Secondly, a subsequent experiment demonstrated that the decline in alcohol preference (volume of alcohol as % of total fluid intake) and in alcohol intake (g/kg) was very slow when hypophysectomy was done after the establishment of a high preference ratio (Finkelberg and Kalant, in preparation).

Addition of LVP treatment to the hypox groups from test Day 10 onwards had the effect of abolishing both surgical and hormonal treatment differences among the groups, since LVP has not only antidiuretic effect, but also behavioral effects similar to those of DGLVP. Yet the groups retained their relative positions as long as the alcohol concentrations remained at the previous FACs. However, when the concentration was raised (Day 17) there was, in effect, a new acquisition phase. The alcohol intakes did not rise as high as in the original acquisition because there was now a choice available. But the important point is that the hypox animals with LVP now behaved in the same way as the sham animals, while they had not done so during the immediately preceding maintenance phase (Days 10–16). This is consistent with the interpretation that DGLVP was acting primarily on acquisition, rather than maintenance, of the alcohol drinking behavior.

A simple speculation about the action of DGLVP and related peptides is that they reduce forgetting between trials in a discrete trial situation. On this basis one might expect improvement even during early acquisition. The demonstration of a peptide-induced improvement in one-trial [32] or first-trial [19] learning is consistent with this suggestion and with the present findings. Inspection of the data shown by Guth *et al.* [17] shows also that, as in the present study, peptide-treated animals exhibit improved acquisition of appetitive behavior beginning early in the training period, and that this effect persists throughout training.

Improvement of acquisition by pituitary peptides has now been shown for active and passive avoidance learning [2, 27, 31, 32], lever pressing for water reward [17], and maze-learning for water [31] and for food [6, 22, 29]. There is growing evidence that pituitary hormones affect attentional processes both in animals and in man [6, 21, 22, 31], which might underlie their apparent (and sometimes contradictory) effects on memory, performance, arousal, learning, and motivation.

It must be emphasized that the present results do not imply that a pituitary mechanism ordinarily governs the development of an alcohol-drinking habit. Alcohol was deliberately chosen for investigation because it does not enhance, indeed in appropriate doses it inhibits, the release of vasopressin and oxytocin. It therefore provides a simpler model for investigating the effects of exogenous peptide hormone on a drug-taking habit.

The levels of alcohol intake observed in the present study do not conform to any accepted definition of alcohol dependence, and probably did not evoke any tolerance or physical dependence. The observed effect of DGLVP is therefore of interest chiefly in relation to the mechanisms governing acquisition or retention of appetitive behaviors. Nevertheless, drug dependence does involve an appetitive behavior. It is therefore tempting to speculate that the relative degree of dependence liabilities of alcohol and other drugs may be related to their respective efficacies in stimulating or inhibiting the release of some of the peptides which can facilitate acquisition of drug self-administration behavior.

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