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Effects of Peptides Related to Neurohypophyseal Hormones on Ethanol Tolerance

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RIGTER, H., C. DORTMANS AND J. C. CRABBE, JR. *Effects of peptides related to neurohypophyseal hormones on ethanol tolerance.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 285–290, 1980.—Mice were rendered tolerant to the hypothermic effect of ethanol by forcing them to inhale ethanol vapor for 3 days. One day after withdrawal, tolerance was assessed by determining the response of the mice to an acute 3 g/kg IP challenge dose of ethanol. Thirty minutes before the injection of ethanol, saline or peptide solution was SC injected. The peptides studied were des-Gly^9-Arg^8-vasopressin (a peptide with reduced peripheral endocrine activities), oxytocin, and analogs and fragments of these peptides. None of the peptides, with the possible exception of oxytocin, affected body temperature in naive animals or the acute hypothermic response to ethanol in non-tolerant mice. Des-Gly^9-Arg^8-vasopressin enhanced the expression of tolerance to ethanol hypothermia; shorter fragments of vasopressin did not share this effect. Oxytocin attenuated the expression of tolerance but this may have been due to an interaction with the acute effects of ethanol.

Vasopressin Oxytocin Tolerance Ethanol Hypothermia

REPEATED, prolonged exposure to alcohol leads to the development of tolerance. Tolerance to alcohol, traditionally considered to be a phenomenon that could be described in purely pharmacologic terms, has recently been demonstrated to be susceptible to behavioral manipulations. This has led to speculation that something analogous to a memory is formed by prior exposure to alcohol; in other words, tolerance to alcohol may be to some degree learned [2, 8, 12].

The neurophypophyseal hormone Arg^8-vasopressin (AVP) is known to enhance learning and memory in a variety of experimental paradigms [1, 5, 14]. A naturally arising question was whether AVP or its structural analogs could affect tolerance to alcohol. Studies in other laboratories and our own work have indicated that AVP and the analogs des-Gly^9-Arg^8-vasopressin (DGAVP) and des-Gly^9-Lys^8-vasopressin (DGLVP) have a variety of effects on alcohol responses.

One group [9,10] rendered mice tolerant to and dependent on alcohol by forcing them to drink a liquid ethanol diet for 7 days. After withdrawal, residual tolerance to alcohol was assessed at 3-day intervals by administering an acute dose of alcohol and measuring the duration of the loss of the righting reflex and hypothermia. Mice were given daily SC injections of saline or 10 μg AVP or oxytocin during the period of induction of tolerance and for 9 days after withdrawal. Animals treated with AVP retained tolerance to ethanol for as long as the peptide was administered. Thereafter, AVP had no residual effect on the rate at which tolerance was lost. AVP was also active when treatment was restricted to the period of testing for tolerance. Oxytocin was ineffective in this study. AVP and oxytocin did not alter blood alcohol levels during the ingestion period or clearance of alcohol after withdrawal or during testing for tolerance. Neither peptide affected sensitivity to the sedative or hypothermic effects of alcohol in non-tolerant mice. It thus appears that vasopressin may retard the decay of tolerance to alcohol. One explanation would be that the peptide improves the retention of a memory associated with tolerance.

In studies of conditioned behavior, AVP has been found both to facilitate acquisition (development) of new responses and to delay extinction (decay) of non-reinforced responses. Both effects have been attributed to a peptide-induced modulation of memory [5,20]. By the same reasoning, one would expect AVP to influence both development and decay of tolerance. This possibility was tested in a series of experiments in our laboratory [15,16]. Alcohol was administered to mice by forcing them to inhale alcohol vapor, with pyrazole given daily to stabilize blood alcohol levels. Residual tolerance to the hypothermic effect of alcohol was assessed one or more days after withdrawal. The efficacy of DGAVP in modulating tolerance was examined by SC implantation of ALZET® osmotic minipumps, from which the peptide was continuously released. By restricting treatment to the period of induction of tolerance or to the period beginning after withdrawal we attempted to dissociate possible peptide effects on development *versus* decay of tolerance. Continuous treatment with DGAVP throughout both the period of induction of tolerance and the period of testing for tolerance

enhanced residual tolerance to the hypothermic effect of the challenge dose of alcohol given one day after withdrawal. When infusion of the peptide was restricted to either the period of induction of tolerance or the period beginning after withdrawal up to the time when tolerance was assessed, only treatment during the development of tolerance clearly enhanced residual tolerance.

Thus, while we found evidence for a facilitatory effect of DGAVP on the development of tolerance, our data failed to clearly confirm the above mentioned finding [9] that AVP also retards the decay of tolerance. We reasoned that procedural differences between the two sets of studies might have caused this discrepancy. In the study of Hoffman *et al.* [9] the peptide was SC injected once daily, whereas in our study the peptide was continuously infused. Therefore, we decided to examine the effect of a single SC injection of peptide on the expression of residual tolerance. In a first set of experiments we found that DGAVP, in a wide dose range, had no effect on body temperature and no effect on the acute hypothermia caused by alcohol. Such control data were also collected for the other peptides studied: the ring amino acid sequence of AVP, pressinoic acid; the tail amino acid sequence of AVP, Pro-Arg-Gly-NH_2; oxytocin; the tail amino acid sequence of oxytocin, Pro-Leu-Gly-NH_2; cyclo-(Leu-Gly); and oxypressin. In a second set of experiments we assessed the efficacy of a single injection of DGAVP to enhance residual tolerance to the hypothermic effect of alcohol. Also, data from preliminary structure-activity studies of tolerance are reported.

METHOD

Male Swiss random-bred mice were purchased from Broekman, Stiphout, The Netherlands. They weighed 20–30 g and were housed with free access to food and water in a room with a 12:12 hr light-dark cycle (lights on at 07:00 a.m.) and controlled temperature (22°C). Testing was performed in the same or in a similar room between 9:30 a.m. and 16:00 p.m.

All peptides used were synthesized by Drs. Greven and Van Nispen (Organon). Des-Gly^9-Arg^8-vasopressin dicitrate (DGAVP) has a blood pressure activity in rats of less than 1.8 mU/mg. The other peptides were Pro-Arg-Gly-NH_2 (PAG), pressinoic acid, oxytocin, Pro-Leu-Gly-NH_2 (PLG), cyclo-(Leu-Gly), and oxypressin. Ethanol and peptides were dissolved in 0.9% saline. Ethanol was given as a 20% (v/v) solution.

Assessment of Effects on Body Temperature and Acute Hypothermia

Rectal temperatures were assessed 5 min before injection of ethanol or saline (baseline) and 45 min after injection. The 45 min treatment-test interval was selected from previous time course studies [4]. Temperature measurements were made with an Ellab DU-3 thermometer, 30 sec after insertion of a probe to a depth of 2.5 cm. In the first experiment, each mouse received two injections, an IP injection with 3 g/kg ethanol or saline, and, within the same minute, a SC injection with 0.1, 1 or 10 μg DGAVP or saline. In other experiments, the SC treatment was given 30–60 min before the IP treatment but, since the results were similar, the data are not reported here. In subsequent studies we tested the other peptides. We selected the highest dose (10 μg) used in tolerance studies. Subcutaneous injection of peptide solution or saline was given 30 min before the IP treatment with ethanol or saline.

Assessment of Effects on Tolerance

Mice were housed in groups of 25 in large plastic cages and made dependent on ethanol using an inhalation method [7]. At 10:00 a.m. on Day 1, a priming dose of ethanol (1.25 g/kg), followed by pyrazole (68.1 mg/kg) was IP injected. Pyrazole treatment was repeated on Day 2 and 3, also at 10:00 a.m. This treatment was used to stabilize blood alcohol levels [7]. The cages housing the mice were placed in a 110×60×40 cm chamber, in which ethanol vapor was continuously led (6–8 mg/l air), using a method described elsewhere [16]. Average mortality during the induction of dependence was 4.6%.

At 9:00 a.m. on Day 4 the animals were withdrawn from ethanol. On Day 5, residual tolerance to the hypothermic effect of an acute challenge dose of 3 g/kg IP ethanol was assessed. Body temperature was measured 5 min before and 45 min after administration of ethanol. For each peptide, a separate study was performed, including 5 groups of tolerant mice and a group of naive mice (control group). The control group was used to assess the acute hypothermic effect of ethanol. The groups of tolerant mice were SC pretreated with saline, 0.01 μg, 0.1 μg or 10 μg peptide, respectively. Pretreatment was 30 min before the injection of ethanol. The control group was pretreated with saline.

RESULTS

Assessment of Effects on Body Temperature and Acute Hypothermia

The results for DGAVP, administered within one minute of saline or ethanol injection, are presented in Table 1. DGAVP had no effect on either the test temperature measured 45 min after treatments or on the difference (Δ), in °C, between baseline and test temperature. Most other peptides studied were also without effect on test temperature or Δ. In separate experiments, groups of 9–11 mice were SC pretreated with saline or peptide. Δ values, across these experiments, ranged from 2.9 ± 0.3°C to 3.5 ± 0.2°C. Comparisons between peptide groups and corresponding saline groups yielded Student's t values of 0.96 for pressinoic acid; 0.24 for PAG; 1.59 for PLG; 0.08 for cyclo-(Leu-Gly); and 0.45 for oxypressin (not significant). Oxytocin did not affect baseline temperature but tended to enhance the acute hypothermic response to ethanol, $t(19)=2.06$, $p<0.1$, and the Δ score, $t(19)=1.93$, $p<0.1$. Oxytocin had no effect on test temperatures in saline-treated mice, $t(17)=0.10$.

Assessment of Effects on Tolerance

As can be seen from Fig. 1, a dose of 0.1 μg DGAVP reduced Δ relative to the saline-treated group, $t(31)=3.67$, $p<0.001$. A similar tendency was seen for the 1 μg dose, $t(32)=1.77$, $p<0.1$. Since all these animals were tolerant, as indicated by reduced Δ's compared with the naive control group (saline-treated tolerant group *versus* control group: $t(37)=2.77$, $p<0.01$), the further reduction of Δ by DGAVP probably reflects enhanced tolerance. DGAVP had no effect on baseline temperatures in this study, indicating again, that the peptide does not affect body temperature *per se*. However, there was a tendency for the tolerant groups of mice to have a slightly lower baseline temperature than the control

TABLE 1

LACK OF EFFECT OF DGAVP ON BODY TEMPERATURE AND ACUTE HYPOTHERMIC ACTION OF ETHANOL

Pretreatment (SC)	Treatment (IP)	n	Temperature (°C) Baseline	Test	Δ	t
Saline	Saline	19	38.2 ± 0.1	38.1 ± 0.1	0.2 ± 0.1	
0.1 μg		16	38.3 ± 0.1	37.9 ± 0.2	0.5 ± 0.1	1.63
1 μg		19	38.0 ± 0.1	37.6 ± 0.1	0.4 ± 0.1	0.94
10 μg		20	38.1 ± 0.1	37.8 ± 0.1	0.3 ± 0.2	0.60
Saline	Ethanol	20	38.3 ± 0.1	35.4 ± 0.2	3.0 ± 0.2	
0.1 μg		19	38.4 ± 0.1	35.2 ± 0.2	3.3 ± 0.2	1.17
1 μg		19	38.2 ± 0.1	35.1 ± 0.2	3.1 ± 0.2	0.47
10 μg		19	38.2 ± 0.1	34.9 ± 0.2	3.3 ± 0.2	1.01

N=number of mice per group. Δ=difference in °C between baseline and test temperatures (mean ± SEM). Student's *t* values given relate to comparisons of Δ values between peptide groups and the corresponding saline-treated group.

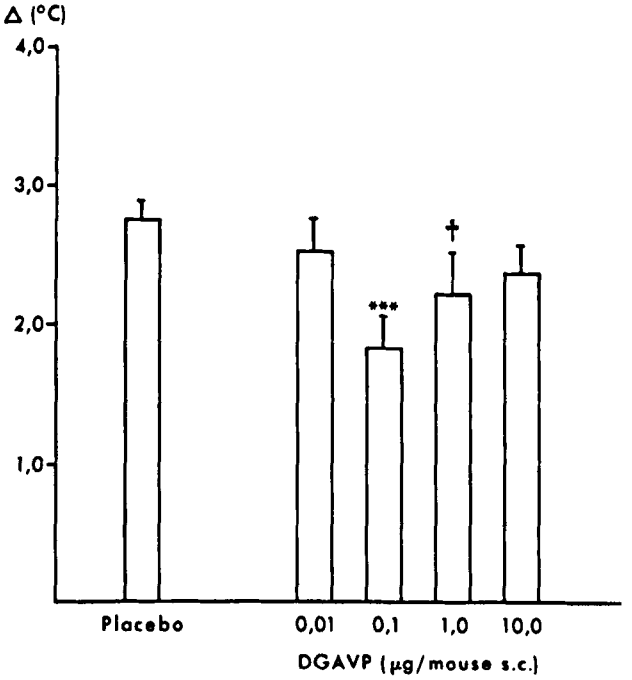

FIG. 1. Effect of a single SC injection of DGAVP on residual tolerance to the hypothermic effect of alcohol. DGAVP or saline was given 30 min before the IP challenge dose of 3 g/kg ethanol, 1 day after withdrawal from chronic ethanol. Δ°C is the difference (mean ± SEM) between baseline rectal temperature, recorded 5 min before acute administration of ethanol, and temperature recorded 45 min after the ethanol treatment. A reduction in Δ°C is thought to reflect increased tolerance. ***$p<0.001$; †$p<0.1$, relative to placebo group (two-tailed Student's *t* test). N=15–17 mice/group. This figure was published in a recent review [14] and is reproduced here with permission of Raven Press.

group, $t(37)=1.94$, $p<0.1$. This has also been observed in other experiments (see Table 2).

The results for the other peptides are summarized in Table 2. The ring and tail amino acid sequences of AVP, pressinoic acid and PAG, did not reduce Δ. Rather, the highest dose of pressinoic acid appeared to increase Δ. Table 2 also shows that the highest dose of oxytocin tested, 10 μg, increased hypothermia and tended to increase Δ. Due to experimental error no data could be collected for the control group in this experiment. However, in a repeat study the existence of tolerance was suggested by a smaller hypothermic response in the saline-treated tolerant group compared with the control group, $t(36)=8.27$, $p<0.001$. The hypothermic response was again increased by 10 μg oxytocin (saline-treated *versus* oxytocin-treated tolerant groups: $t(35)=2.04$, $p<0.05$), although not sufficiently to approach the values measured for the control group (oxytocin-treated tolerant group *versus* control group: $t(37)=4.09$, $p<0.001$). Table 2 further shows that the other peptides studied (PLG, cyclo-(Leu-Gly) and oxypressin) did not affect any of the measures used.

DISCUSSION

We previously reported that DGAVP enhances tolerance to the hypothermic of alcohol, when peptide treatment was restricted to the period of induction of tolerance. Continuous infusion of DGAVP did not clearly influence our measure of tolerance when infusion was restricted to the period of testing for tolerance [16]. However, the present data indicate that a single injection of DGAVP shortly before testing for tolerance reduces the hypothermic response to a challenge dose of alcohol. We believe that this effect reflects a modulation of the expression of functional tolerance. Previous studies [10,16] from our and other laboratories have well characterized the reduction of ethanol-induced hypothermia seen in animals previously subjected to chronic treatment with alcohol. These studies have indicated that this reduced response cannot be attributed to possible changes in the metabolism of alcohol. The existence of functional tolerance is further suggested by the finding of a parallel shift of the dose-response curve of alcohol (Rigter, unpublished findings).

Our finding that DGAVP enhanced the expression of residual tolerance to alcohol when the peptide was given exclusively during the period of testing for tolerance seems consistent with a similar finding of Hoffman *et al.* [9] with AVP. We did not report here data for AVP since we have found that, with the short treatment-test intervals used in our

TABLE 2

EFFECT OF PEPTIDES ON TOLERANCE TO THE HYPOTHERMIC EFFECT OF ETHANOL

Pretreatment (SC)	Condition	n	Temperature (°C) Baseline	Test	Δ	t
Saline	Tolerant	19	38.3 ± 0.1	36.6 ± 0.1	1.7 ± 0.1	
PA 0.01 μg		20	38.2 ± 0.1	36.4 ± 0.1	1.8 ± 0.1	0.45
PA 0.1 μg		17	38.4 ± 0.1	36.3 ± 0.2	2.1 ± 0.2*	1.77
PA 1 μg		19	38.3 ± 0.1	36.3 ± 0.2	2.0 ± 0.2	1.22
PA 10 μg		19	38.3 ± 0.1	36.1 ± 0.2‡	2.2 ± 0.2†	2.47
Saline	Control	10	38.6 ± 0.1†	35.7 ± 0.2‡	2.9 ± 0.3§	4.18
Saline	Tolerant	18	38.2 ± 0.1	36.7 ± 0.1	1.5 ± 0.2	
PAG 0.01 μg		17	38.2 ± 0.1	36.5 ± 0.1	1.6 ± 0.2	0.58
PAG 0.1 μg		19	38.2 ± 0.1	36.5 ± 0.1*	1.7 ± 0.2	1.15
PAG 1 μg		18	38.0 ± 0.2	36.6 ± 0.2	1.3 ± 0.2	0.67
PAG 10 μg		18	38.2 ± 0.1	36.8 ± 0.1	1.3 ± 0.2	0.57
Saline	Control	9	38.5 ± 0†	35.2 ± 0.2§	3.4 ± 0.2§	6.96
Saline	Tolerant	14	38.5 ± 0.1	36.4 ± 0.2	2.1 ± 0.2	
OT 0.01 μg		15	38.5 ± 0.1	36.2 ± 0.2	2.2 ± 0.2	0.43
OT 0.1 μg		14	38.6 ± 0.2	36.2 ± 0.3	2.5 ± 0.3	0.91
OT 1 μg		15	38.7 ± 0.1	36.2 ± 0.2	2.5 ± 0.3	1.24
OT 10 μg		16	38.4 ± 0.1	35.8 ± 0.2†	2.6 ± 0.2*	1.80
Saline	Tolerant	19	38.3 ± 0.1	36.4 ± 0.2	1.9 ± 0.2	
PLG 0.01 μg		21	38.0 ± 0.1*	36.1 ± 0.1	1.9 ± 0.2	0.09
PLG 0.1 μg		19	38.3 ± 0.1	36.4 ± 0.2	1.9 ± 0.2	0.08
PLG 1 μg		20	38.2 ± 0.1	36.4 ± 0.2	1.8 ± 0.1	0.78
PLG 10 μg		20	38.4 ± 0.1	36.5 ± 0.1	1.9 ± 0.1	0.05
Saline	Control	11	38.4 ± 0.1	35.3 ± 0.1§	3.1 ± 0.2§	4.48
Saline	Tolerant	15	38.4 ± 0.1	36.9 ± 0.2	1.6 ± 0.2	
cLG 0.01 μg		17	38.6 ± 0.2	37.0 ± 0.1	1.6 ± 0.1	0.05
cLG 0.1 μg		17	38.2 ± 0.1	36.8 ± 0.1	1.5 ± 0.1	0.46
cLG 1 μg		19	38.4 ± 0.2	36.9 ± 0.1	1.5 ± 0.1	0.29
cLG 10 μg		20	38.2 ± 0.2	36.9 ± 0.2	1.3 ± 0.1	1.31
Saline	Control	20	38.7 ± 0.1†	35.7 ± 0.2§	3.0 ± 0.3§	4.30
Saline	Tolerant	20	38.6 ± 0.1	36.5 ± 0.2	2.1 ± 0.2	
OP 0.01 μg		20	38.5 ± 0.1	36.5 ± 0.1	2.1 ± 0.2	0.08
OP 0.1 μg		19	38.7 ± 0.1	36.5 ± 0.2	2.2 ± 0.2	0.52
OP 1 μg		20	38.6 ± 0.1	36.4 ± 0.1	2.2 ± 0.1	0.61
OP 10 μg		20	38.7 ± 0.1	36.2 ± 0.2	2.5 ± 0.2	1.37
Saline	Control	20	38.5 ± 0.1	35.5 ± 0.2§	3.0 ± 0.2§	3.67

All animals were IP injected with 3 g/kg ethanol. Pretreatment was 30 min before ethanol. N=number of mice per group. Data are means ± SEM. Δ=difference in °C between baseline and test temperatures. Student's *t* values given relate to comparisons of Δ scores between a given group and the corresponding saline-treated tolerant group of mice. *$p<0.1$; †$p<0.05$; ‡$p<0.01$; §$p<0.001$ (two-tailed Student's *t* test). PA=pressinoic acid; PAG=Pro-Arg-Gly-NH_2; OT=oxytocin; PLG=Pro-Leu-Gly-NH_2; cLG=cyclo(Leu-Gly); OP=oxypressin.

studies, AVP affects baseline temperatures. Although the data from our AVP studies would be consistent with a peptide-induced enhancement of tolerance, the effect of AVP on body temperature may have confounded the measurement of tolerance. DGAVP, on the other hand, has greatly reduced peripheral endocrine activities, and, perhaps for that reason, has no effect on body temperature and on the acute hypothermia caused by alcohol.

The measure of tolerance used in the present studies was attenuation of hypothermia produced by alcohol. We intend to examine other measures of tolerance, too. It should be noted that the response measure used seems not to be crucial since AVP has been found by Hoffman *et al.* to enhance tolerance to both the hypothermic effect and the sedative effect of alcohol [10]. We have found no tolerance to the sedative effect of alcohol under the conditions used in our studies. This, together with evidence that tolerance to the hypothermic effect of alcohol is lost more rapidly in our mice than in those of Hoffman *et al.* [10,16], indicates that the degree of tolerance induced in our studies is less than in those of Hoffman *et al.* This suggests that the facilitatory effect of vasopressin-like peptides on tolerance may be independent of the degree of tolerance present. Nevertheless, we feel that in future studies the degree of tolerance and the patterning of peptide treatment should be varied systematically. From the available data, we cannot determine whether AVP or DGAVP affect the rate of decay of tolerance or the degree of tolerance displayed at a given test. Such studies would also bear on the possibility that there are learning components in tolerance (see Introduction). For example, vasopressin is known to enhance performance of a previously learned response when the peptide is given shortly before the retention test. This effect has been attributed to facilitated memory retrieval [20]. If tolerance contains learned components, this may explain the ability of vasopressin-like peptides to enhance residual tolerance. However, we have argued elsewhere [15] that it is difficult to see how the mice in our experiments could have learned discrete alcohol-related stimulus-response associations since alcohol was administered continuously during the induction of tolerance.

Nevertheless, there is a parallel between the effects of AVP and DGAVP on tolerance to alcohol and the effects of these peptides on conditioned behavior. Does this analogy also extend to oxytocin? Oxytocin has been reported to have effects on learning and memory which, under specific conditions, are opposite to those of vasopressin-like peptides [1,17]. The data reported here suggest that oxytocin enhances the acute hypothermic effect of alcohol. Our results therefore do not allow the conclusion that oxytocin and vasopressin may also have an opposite effect on tolerance to alcohol hypothermia: attenuation *versus* enhancement. Others have found no effect of oxytocin on tolerance to alcohol or on the acute hypothermic effect of alcohol [10]. An apparent difference between the two sets of studies is the duration of the treatment-test interval. Hoffman *et al.* administered the peptide approximately 17 hr before daily tests while our animals were injected with oxytocin shortly before testing.

The results of our preoiminary structure-activity studies suggest that the activity of DGAVP may be lost or reduced when this peptide is fragmented. The ring and tail amino acid sequences of AVP did not enhance tolerance to alcohol in our paradigm. The structure-activity relationship for behavioral effects of vasopressin depend upon the test used. In tests of memory consolidation intracerebroventricularly injected pressinamide is more active than PAG [5] while in tests of morphine dependence the activity of vasopressin appears to reside in PAG [21]. There was an indication in our data that SC injected pressinoic acid may attenuate tolerance to the hypothermic effect of alcohol As yet, this is an incidental finding that needs to be replicated. At any rate, there was no sign of enhanced tolerance. We have not yet tested the related peptide, pressinamide. In the study reported here, PAG was inactive; we have confirmed this lack of activity in repeat studies.

A number of peptides structurally related to vasopressin and oxytocin have been studied in tests for tolerance to opiates/opioids. DGAVP enhanced the expression of tolerance to the induction of stereotyped behavior by morphine in cats [3] or to an analgetic effect of morphine in mice [11] or rats [6]. This seems to be consistent with the findings reported for alcohol tolerance. PLG has been reported to enhance tolerance to an analgetic effect of morphine [18] or β-endorphin [20] in rats, although a recent attempt at replication has been unsuccessful [13]. In mice, PLG (and cyclo(Leu-Gly)) were found to attenuate tolerance to morphine [22]. Both peptides were inactive in our test for alcohol tolerance. It thus appears that peptides may enhance or attenuate tolerance to various substances, the structure-activity relationships and the direction of effect being highly dependent on procedural details. Most importantly, however, there is now considerable evidence that naturally occurring peptides may modulate the development and decay of tolerance and other adaptive responses. This suggests that these peptides, notably those related to neurophypophyseal hormones, play a physiological role in CNS adaptability.

REFERENCES

1. Bohus, B., I. Urban, T. B. van Wimersma Greidanus and D. de Wied. Opposite effects of oxytocin and vasopressin on avoidance behavior and hippocampal theta rhythm in the rat. *Neuropharmacology* **17**: 239–247, 1978.
2. Cohen, M., A. S. Keats, W. A. Krivoy and G. Ungar. Effect of actinomycin on morphine tolerance. *Proc. Soc. exp. Biol.* **119**: 381–384, 1965.
3. Cools, A. R., C. L. E. Broekkamp, L. C. M. Gieles, A. Megens and H. J. G. M. Mortiaux. Site of action of development of partial tolerance to morphine in cats. *Psychoneuroendocrinology* **2**: 17–33, 1977.
4. Crabbe, J. C., H. Rigter, J. Uijlen and C. Strijbos. Rapid development of tolerance to the hypothermic effect of ethanol in mice. *J. Pharmac. exp. Ther.* **208**: 128–133, 1979.
5. De Wied, D. Behavioral effects of intraventricularly administered vasopressin and vasopressin fragments. *Life Sci.* **19**: 685–690, 1976.
6. De Wied, D. and W. H. Gispen. Impaired development of tolerance to morphine analgesia in rats with hereditary diabetes insipidus. *Psychopharmacologia* **46**: 27–29, 1976.
7. Goldstein, D. and N. Pal. Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. *Science* **172**: 288–289, 1971.
8. Hinson, R. E. and S. Siegel. The contribution of Pavlovian conditioning to ethanol tolerance and dependence. In: *Alcohol Tolerance and Dependence*, edited by H. Rigter and J. C. Crabbe. Amsterdam: Elsevier/North-Holland Biomedical Press, 1980, pp. 179–197.

9. 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.
10. Hoffman, P. L., R. F. Ritzmann, R. Walter and B. Tabakoff. Arginine vasopressin maintains ethanol tolerance. *Nature* **276**: 614–616, 1978.
11. Krivoy, W. A., E. Zimmermann and S. Lande. Facilitation of development of resistance to morphine analgesia by desglycinamide^9-lysine vasopressin. *Proc. natn. Acad. Sci. U.S.A.* **71**: 1852–1856, 1974.
12. LeBlanc, A. E., C. X. Poulos and H. D. Cappell. Tolerance as a behavioral phenomenon: evidence from two experimental paradigms. In: *Behavioral Tolerance: Research and Treatment Implications*, edited by N. A. Krasnegor. NIDA Research Monograph 18, Washington: U.S. Government Printing Office, 1978, pp. 72–89.
13. Mucha, R. F. and H. Kalant. Failure of prolyl-leucyl-glycinamide to alter analgesia measured by the Takemori test in morphine-pretreated rats. *J. Pharm. Pharmac.* **31**: 572–573, 1979.
14. Rigter, H. and J. C. Crabbe. Modulation of memory by pituitary hormones and related peptides. *Vitams Horm.* **37**: 153–241, 1979.
15. Rigter, H. and J. C. Crabbe. Alcohol: modulation of tolerance by neuropeptides. In: *The Psychopharmacology of Alcohol*, edited by M. Sandler. New York: Raven Press, 1980, pp. 179–189.
16. 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. Pharmac.* **64**: 53–68, 1980.
17. Schulz, H., G. L. Kovács and G. Telegdy. Effect of physiological doses of vasopressin and oxytocin on avoidance and exploratory behavior in rats. *Acta physiol. hung.* **45**: 211–215, 1974.
18. Székely, J. L., E. Miglécz, Z. Dunai-Kovács, I. Tarnawa, A. Z. Rónai, L. Gráf and S. Bajusz. Attenuation of morphine tolerance and dependence by α-melanocyte stimulating hormone (α-MSH). *Life Sci.* **24**: 1931–1938, 1979.
19. Van Ree, J. M., B. Bohus, D. H. G. Versteeg and D. de Wied. Neurohypophyseal principles and memory processes. *Biochem. Pharmac.* **27**: 1793–1800, 1978.
20. Van Ree, J. M. and D. de Wied. Neurohypophyseal hormones and morphine dependence. In: *Opiates and Endogenous Opioid Peptides*, edited by H. W. Kosterlitz. Amsterdam: Elsevier/North-Holland, 1976, pp. 443–445.
21. Van Ree, J. M. amd D. de Wied. Effect of neurophypophyseal hormones on morphine dependence. *Psychoneuroendocrinology* **2**: 35–41, 1977.
22. Walter, R., R. F. Ritzmann, H. N. Bhargava and L. B. Flexner. Prolyl-leucyl-glycinamide, cyclo (leucylglycine), and derivatives block development of physical dependence on morphine in mice. *Proc. natn. Acad. Sci. U.S.A.* **76**: 518–520, 1979.