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Vasopressin-Like Peptides Retain Ethanol Tolerance in the Absence of Changes in Serotonin Synthesis in Limbic Structures

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SPEISKY, M. B. AND H. KALANT. *Vasopressin-like peptides retain ethanol tolerance in the absence of changes in serotonin synthesis in limbic structures.* PHARMACOL BIOCHEM BEHAV **25**(4) 797–803, 1986.—Central tolerance to the effects of ethanol in rats can be prolonged beyond its normal time of disappearance by administration of vasopressin (AVP) or desglycinamide-arginine-vasopressin (DGAVP) after ethanol withdrawal. While the mechanism underlying this effect is unknown, we have reported that specific depletion of hippocampal serotonin (5-HT) prevents the prolongation of tolerance by DGAVP. The present study explored possible presynaptic interactions between DGAVP and 5-HT terminals in the hippocampus, in relation to tolerance retention. When administered acutely, DGAVP had no effect on the rates of hippocampal or septal 5-HT synthesis in naive rats, as assessed by the NSD 1015 method. Moreover, chronic DGAVP treatment that maintained tolerance did not change the *in vivo* rate of 5-HT synthesis in the hippocampus or septum. Similarly, no significant differences were found in the levels of hippocampal 5-HT or 5-HIAA. Septal 5-HIAA levels were slightly but significantly lower in ethanol-DGAVP than in ethanol-saline rats. *In vitro* studies revealed, on the other hand, that addition of AVP to the incubation medium failed to affect the spontaneous and stimulated release of endogenous 5-HT from hippocampal slices. While the lack of changes in hippocampal 5-HT synthesis argues against a presynaptic DGAVP-5-HT interaction, the possibility remains of a peptide modulation of 5-HT postsynaptic actions.

Ethanol tolerance Vasopressin DGAVP 5-HT synthesis Hippocampus

ADMINISTRATION of arginine vasopressin (AVP) or desglycinamide[9]-arginine[8]-vasopressin (DGAVP, an analog almost devoid of endocrine activity) to rodents has been reported by several groups to maintain tolerance to several effects of ethanol after ethanol administration is discontinued [8, 11, 16, 18]. Although at present little is known about the neuronal systems involved in this peptide-induced maintenance of tolerance, indirect evidence supports the involvement of the central serotonergic [11] and noradrenergic systems [7] in rats and mice, respectively. A role for the serotonin (5-HT) system was suggested by the observation that electrolytic lesion of the median raphe nucleus (but not the dorsal or magnus) interfered with the ability of DGAVP to maintain tolerance to ethanol-induced hypothermia and motor impairment in rats [11]. This observation led to the hypothesis that a DGAVP-5-HT interaction had to take place, at some point in the mesolimbic serotonergic pathway, for this peptide to maintain ethanol tolerance.

Further support for this idea was provided by our finding that the specific destruction of serotonergic fibers innervating the hippocampus totally prevented DGAVP from maintaining tolerance to ethanol-induced motor impairment [18]. That the putative peptide-5-HT interaction could take place at the level of the hippocampus is also indirectly supported by the report [1] that addition of AVP *in vitro* increased the synthesis and potassium-stimulated release of endogenous 5-HT in the hippocampal slice. In addition, the presence of synaptic AVP terminals [2] and specific receptors [15] in the hippocampus is consistent with the concept that this region could constitute one of the possible anatomical sites involved in the central actions of vasopressin-like peptides.

In the present study we explored possible presynaptic interactions between DGAVP and 5-HT terminals. Specifically, the effects of acute and chronic DGAVP administration on the *in vivo* rate of 5-HT synthesis in hippocampus and septum were studied in relation to the peptide-induced main-

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tenance of tolerance. In addition, attempts were made to replicate the observation by Auerbach and Lipton [1] that AVP increases the release of endogenous 5-HT from the hippocampal slice.

METHOD

Animals

Male Wistar rats (Charles River, Montréal, Canada), weighing 280–300 g when purchased, were individually housed in an environmentally controlled room (21–23°C and 40% relative humidity) with lighting on from 0700 to 1900 hr. In the acute and *in vitro* studies rats were kept in the animal quarters for one week prior to the experiment. Water and standard Purina rat chow were available ad lib, except for the night prior to the experiment when rats were fasted while water continued to be available. In the chronic studies standard Purina rat chow and water were available ad lib until the rats reached 330 g. They were held at this body weight for at least one week prior to the experiment by appropriate restriction of the daily chow ration. Rats were fasted overnight prior to the experiment, while water continued to be available.

Materials

Sources. Noradrenaline (NA) bitartrate, dopamine hydrochloride, 5-HT creatinine sulfate, 5-hydroxyindoleacetic acid (5-HIAA), 5-hydroxytryptophan (5-HTP), dihydroxybenzylamine (DHBA), m-hydroxybenzylhydrazine dihydrochloride (NSD 1015), imipramine·HCl and AVP (acetate salt, grade VIII, 390 I.U./mg) were purchased from Sigma Chemicals (St. Louis, MO). DGAVP was kindly donated by Organon (Oss, The Netherlands) through the courtesy of Dr. H. Greven. Purity control of DGAVP was carried out by high voltage paper electrophoresis as previously described [4] using a solvent system of pyridine:acetic acid:water (50:1:950 v/v) buffered at pH 6.5, at 2000 V for 1 hr.

KRP buffer. The composition of the Krebs-Ringer-phosphate (KRP) buffer was: 128 mM NaCl, 4.8 mM KCl, 0.75 mM $CaCl_2$, 1.2 mM $MgSO_4$, 16 mM glucose, 16 mM Na_2HPO_4 (pH 7.4), 10^{-2} mM imipramine. KRP-high K^+ buffer was made by replacing a portion of the NaCl with an equimolar amount of KCl to a final concentration of 55 mM KCl. Imipramine was always included in the buffer in order to inhibit the neuronal uptake of the endogenous 5-HT previously released into the incubation medium.

Measurement of rate of 5-HT synthesis and endogenous monoamine levels. The rate of 5-HT synthesis in discrete regions of the brain was estimated by measuring the *in vivo* accumulation of the serotonin precursor 5-HTP, after inhibition of the aromatic amino acid decarboxylase (E.C.4.1.1.28) with NSD 1015 as described by Carlsson *et al.* [3].

NSD 1015 (200 mg/kg in saline) was injected intraperitoneally. Thirty min later, the rats were decapitated and the septa and hippocampi were immediately dissected out. The tissue samples were frozen in dry ice and kept at −70°C until assayed. 5-HTP was measured in acidic extracts of brain samples by high performance liquid chromatography (HPLC) with electrochemical detection, according to the method described for monoamines [9]. In this instance, the mobile phase contained a higher concentration (0.75 mM) of the ion pairing reagent octyl sodium sulfate (OSS) in order to resolve the 5-HTP signal from that of NA. All other parameters were kept unchanged [9]. This method permitted the simultaneous determination of 5-HTP, NA, 5-HIAA, dopamine and 5-HT. Possible interference by DOPA, MHPG, HVA and NSD 1015 was ruled out in preliminary experiments. Standards were prepared in 0.1 M perchloric acid (PCA) containing 0.025 mM ascorbic acid, and stored at −70°C.

PCA extracts of brain samples were prepared by homogenizing the tissue in 10–20 volumes of 0.1 M PCA containing 0.025 mM ascorbic acid and 40 ng/ml of DHBA as internal standard. Homogenates were prepared with a Polytron (Brinkmann Instruments, New York) probe PT 10 TS, at setting 5 for 20 sec, and centrifuged at 30,000 × g for 20 min in a Sorvall Model RC-2B refrigerated centrifuge. The supernatants were separated and immediately injected into the HPLC or stored at −70°C to be processed within 2 weeks.

Moving Belt Test

Training period. In this test, rats were trained to walk on a motor-driven belt that moved continuously over a shock grid [13]. When the rat stepped off the belt, it received a shock and a cumulative timer was activated to measure total time off belt during the 2-min trial.

Seventy rats were trained to a criterion of 99% correct performance (i.e., not more than 1.2 sec off belt during any 2-min trial). Training sessions began within the first week after arrival of the animals in the vivarium.

Test session. On test days, the motor impairment was measured for each rat in 4 consecutive trials starting at 7, 17, 27 and 47 min after an intraperitoneal (IP) injection of a test dose of ethanol (2.3 g/kg as an 11% w/v solution in saline).

Upon completion of the last trial, 50 μl of blood was taken from the cut tip of the tail for gas chromatographic determination of blood ethanol concentration [12]. Rats were run blindly with respect to their chronic treatments.

Acute Studies

To assess the effects of the acute administration of DGAVP on the rate of *in vivo* accumulation of 5-HTP in the brain, DGAVP (10 μg/rat in 0.2 ml of saline) or saline was given subcutaneously (SC) in the back of the neck to rats fasted overnight. NSD 1015 (200 mg/kg in saline) was injected intraperitoneally either immediately following (Experiment 1) or 3.5 hr after (Experiment 2) the DGAVP or saline injection. Rats were sacrificed by decapitation 30 min after the administration of NSD 1015 (i.e., between 10:00 and 12:00 hr in Experiment 1 and 13:00 and 15:00 hr in Experiment 2) and the rates of 5-HT synthesis were measured in the septum and hippocampus as described above.

Chronic Studies

After an initial test of ethanol-induced motor impairment on the moving belt, the rats were subdivided into 2 groups, matched according to their initial impairment scores, to receive either sucrose (n=28) or ethanol treatment (n=42).

Ethanol was administered to rats by gavage as a 25% w/v solution (in tap water), starting with a daily dose of 3 g/kg that was gradually increased up to 5 g/kg by 0.5 g/kg increments every 2 days. Control rats received chronic equicaloric sucrose treatment. Chronic treatment lasted for 26 days, with tolerance being measured on days 12 and 26 of

TABLE 1

ACCUMULATION OF 5-HTP AND BRAIN MONOAMINE LEVELS AFTER INJECTION OF NSD 1015

Group	5-HTP (ng/g)	5-HT (ng/g)	5-HIAA (ng/g)	NA (ng/g)
A. Hippocampus				
Saline—0 min	N.D.	313 ± 15	369 ± 30	793 ± 25
Saline—30 min	N.D.	314 ± 16	366 ± 14	819 ± 42
NSD-1015—0 min	N.D.	281 ± 29	363 ± 14	837 ± 41
NSD-1015—30 min	172 ± 18†	314 ± 20	235 ± 11*	907 ± 17
B. Septum				
Saline—0 min	N.D.	635 ± 69	471 ± 66	1673 ± 163
Saline—30 min	N.D.	601 ± 63	457 ± 42	1929 ± 100
NSD-1015—0 min	N.D.	571 ± 111	455 ± 70	1710 ± 145
NSD-1015—30 min	223 ± 23†	462 ± 75	245 ± 19*	1601 ± 330

Rats injected with NSD 1015 (200 mg/kg, IP) or saline were sacrificed by decapitation immediately or 30 min after receiving their respective injections. The hippocampi and septa were rapidly dissected out, frozen on dry ice and kept at −70° C until assayed by HPLC-EC. Results are expressed as ng/g wet weight of tissue. N.D. = not detected.

*Designates $p<0.01$.

†To convert these values to rates of 5-HT synthesis, multiply × 2 to obtain a corresponding accumulation for 1 hr instead of 30 min, and divide by the molecular weight of 5-HTP (220.22). This will yield a rate expressed in nanomoles/g/hr. For the values shown, the corresponding rates are 1.6 nmol/g/hr for hippocamus and 2.0 nmol/g/hr for septum.

treatment. Upon ethanol and sucrose withdrawal, each group was divided into two equal subgroups, matched according to their impairment scores on day 26, to receive either saline or DGAVP treatment for 33 days. DGAVP (10 μg/rat daily, freshly dissolved in 0.2 ml of 0.9% saline) was administered to rats SC in the back of the neck, between 10:00 and 12:00 hr on treatment days. On test days, the peptide was given approximately 2 hr after the testing was finished. Control rats received SC injections of 0.2 ml of 0.9% saline solution.

Tolerance was measured on days 12, 26 and 33 of this treatment. Approximately 24 hr after the last DGAVP or saline injection, the rats were given an IP injection of NSD 1015 (200 mg/kg), and sacrificed 30 min later. Their hippocampi and septa were immediately dissected out, frozen on dry ice and stored at −70°C for HPLC-EC analysis of their 5-HTP and monoamine content.

In Vitro *Studies*

Measurement of endogenous 5-HT release. Transverse hippocampal slices (300 μm in thickness), prepared with a McIlwain tissue chopper, were obtained from each hippocampus (approximately 50 mg), suspended in 0.5 ml of oxygenated KRP buffer (pH 7.4) and preincubated for 5 min at 37°C. Upon completion of the preincubation period, the slices were spun down at 15,000 × g for 1 min in an Eppendorf centrifuge. The supernatant medium was discarded and the slices were resuspended in fresh oxygenated buffer (either normal KRP or KRP-high K^+) and incubated for 5 min at 37°C. To study the effects of AVP on the basal and K^+-induced release of 5-HT, different amounts of AVP were added to both the preincubation and incubation media. The peptide was freshly dissolved in 0.1 mM HCl and diluted into the preincubation and incubation media to yield final concentrations of 10^{-7} and 10^{-6} M. For the control slices, 0.1 mM HCl was used in the same dilutions but without peptide.

Both basal and K^+-induced release of 5-HT were studied in tissue obtained from the same animal. For this purpose, for each rat, one hippocampus was incubated in normal KRP, while the other was incubated in KRP-high K^+.

The incubation was terminated by centrifugation at 15,000 × g for 1 min. The supernatant was aspirated and a 400 μl sample was transferred to an Eppendorf tube containing 100 μl of an acid solution consisting of 0.1 M PCA, 0.025 mM ascorbic acid and 0.7 μM DHBA; 50 or 100 μl aliquots of this acidified incubation medium were injected directly into the HPLC to quantify the released monoamines, as described above.

Statistical Analysis

Results are expressed as group means±standard error of the mean (SEM). For some statistical comparisons, the two-tailed Student's *t*-test for unpaired data [17] was used. Probability (*p*) values equal to or lower than 0.05 were considered to indicate statistical significance. When appropriate, the data were subjected to analysis of variance using a computer package program (BMDP statistical software, University of California).

RESULTS

Acute Studies

Administration of NSD 1015 resulted in the accumulation of measurable amounts of 5-HTP in both the hippocampus and septum, but had no significant effect on the steady state levels of 5-HT or NA in either region (Table 1). Rats sac-

TABLE 2

EFFECT OF ACUTE DGAVP ADMINISTRATION ON THE RATE OF TRYPTOPHAN HYDROXYLATION AND MONOAMINE LEVELS IN THE HIPPOCAMPUS AND SEPTUM

Group	5-HTP (ng/g/30 min)	5-HT (ng/g)	5-HIAA (ng/g)	NA (ng/g)
		Experiment 1		
A. Hippocampus				
Saline	164 ± 10	332 ± 31	274 ± 20	976 ± 33
DGAVP	158 ± 8	336 ± 11	264 ± 10	968 ± 52
B. Septum				
Saline	253 ± 32	511 ± 60	284 ± 26	1634 ± 143
DGAVP	269 ± 10	554 ± 22	272 ± 13	1764 ± 119
		Experiment 2		
A. Hippocampus				
Saline	83 ± 9	319 ± 23	207 ± 3	883 ± 13
DGAVP	93 ± 11	328 ± 22	212 ± 15	840 ± 47
B. Septum				
Saline	196 ± 16	721 ± 31	278 ± 13	2001 ± 160
DGAVP	180 ± 13	651 ± 19	271 ± 17	1983 ± 123

Rats were given a SC injection of DGAVP (10 μg/rat) or saline immediately prior (Experiment 1) or 3.5 hr after (Experiment 2) the administration of NSD 1015 (200 mg/kg, IP). Thirty min later rats were sacrificed by decapitation and their hippocampi and septa were rapidly dissected out, frozen on dry ice and kept at −70° C until assayed by HPLC-EC, n=6 per group.

rificed 30 min after administration of NSD 1015 presented levels of 5-HTP that corresponded to a calculated synthesis rate of 1.6 and 2.0 nmol/g/hr in the hippocampus and septum respectively (Table 1). In addition, this group presented significantly decreased levels of 5-HIAA in both brain regions relative to the control group.

Comparison of 5-HTP levels in the hippocampus and septum of DGAVP and saline-treated rats (Table 2, Experiment 1) indicates that the peptide administration 30 min prior to sacrifice did not alter the rate of tryptophan hydroxylation in these regions. Hippocampal and septal levels of 5-HT, 5-HIAA and NA were also unaffected by the administration of DGAVP.

Administration of DGAVP 4 hr prior to sacrifice (Table 2, Experiment 2), proved equally ineffective in promoting changes in the rate of 5-HT synthesis or levels of endogenous monoamines, either in the septum or hippocampus.

For a reason at present unclear, the rates of tryptophan hydroxylation were markedly lower in Experiment 2 relative to those found in Experiment 1 (see Table 2). While it is known that 5-HT synthesis is subject to circadian variation [6] this difference is unlikely related to it since in both experiments rats were sacrificed during the light cycle, with a difference of no more than 3 hr (see the Method section, Acute Studies).

Chronic Studies

After 26 days of chronic treatment (Fig. 1), the ethanol-receiving group displayed a significant degree of tolerance as evidenced by the smaller area under the curve of time-off-belt versus time, relative to that of the sucrose controls, $F(1,56)=30.18$, $p<0.00001$. Thirty-three days after ethanol withdrawal, tolerance to the ethanol-induced motor impairment had completely disappeared in the rats that received saline during the withdrawal period (Fig. 2). Administration of DGAVP, on the other hand, resulted in the retention of central tolerance to ethanol, indicated by consistently smaller impairment scores relative to those of the controls, $F(1,21)=6.18$, $p=0.0214$, in the absence of changes in blood alcohol concentrations (see legend to Fig. 2).

Analysis of the rate of *in vivo* hydroxylation of tryptophan in both the hippocampus and the septum (Table 3) showed no significant differences between the 4 treatment groups. Therefore, the ability of chronic DGAVP treatment to maintain ethanol tolerance is unlikely to be related to a change in the total synthesis of 5-HT in the presynaptic nerve terminals of the hippocampus or septum. In addition, no differences were found in the levels of 5-HT, 5-HIAA or NA between the different groups in either the hippocampus or the septum. 5-HIAA levels, however, were slightly lower in the septum of ethanol-DGAVP rats relative to those of ethanol-saline controls.

In Vitro *Studies*

As shown in Fig. 3, incubation of slices in high K^+-KRP buffer resulted in a 3- to 4-fold increase in the amount of endogenous 5-HT released, in comparison to the amount released under basal conditions. In a calcium-free medium, however, high K^+ concentrations failed to promote release of 5-HT above basal levels (data not shown).

Addition of AVP (10^{-7} or 10^{-6} M) to the incubation media promoted no significant changes in either the basal or the K^+-stimulated release of endogenous 5-HT from the hippocampal slice.

DISCUSSION

The present study addresses the effects of acute and

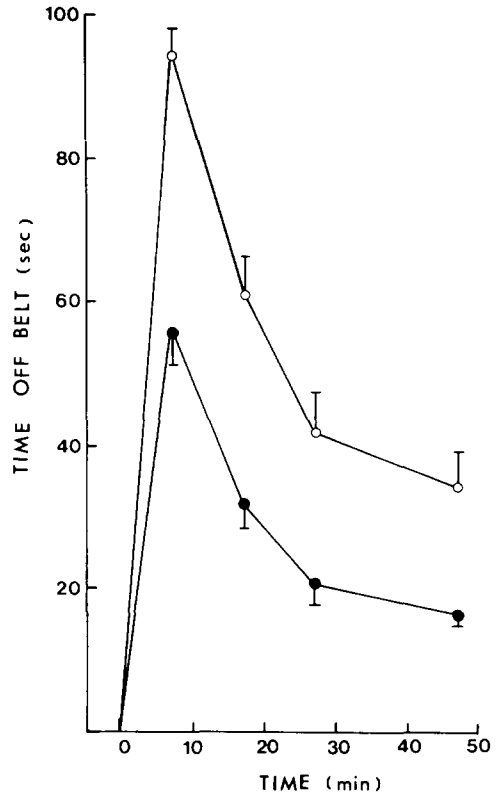

FIG. 1. Time course of the ethanol-induced motor impairment, tested after 26 days of chronic treatment. Time off belt (sec) was recorded 7, 17, 27 and 47 min after the injection of a test dose of ethanol (2.0 g/kg, IP). Plotted values are group means; vertical bars represent positive or negative half of the respective SEM. ○ sucrose; ● ethanol.

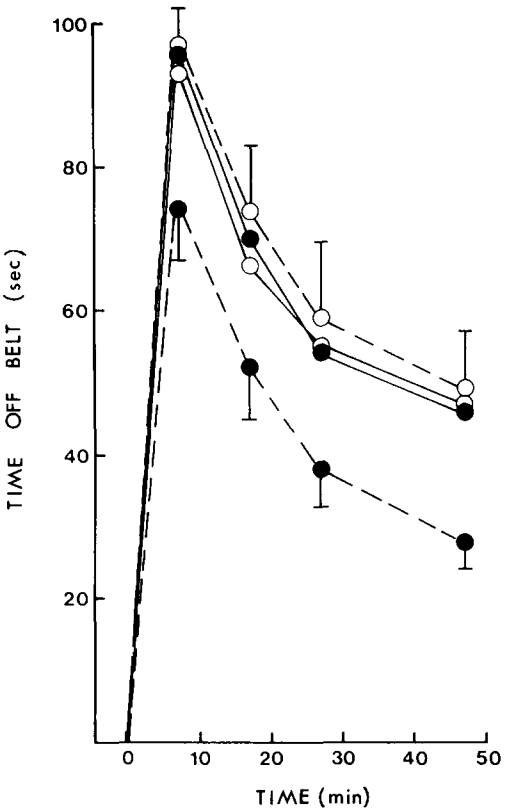

FIG. 2. Time-course of the ethanol-induced motor-impairment, tested 33 days after ethanol withdrawal. Time off belt (sec) was recorded 7, 17, 27 and 47 min after the injection of a test dose of ethanol (2.3 g/kg, IP). Results are plotted as in Fig. 1. ○——○ sucrose-saline; ○— —○ sucrose-DGAVP; ●——● ethanol-saline; ●— —● ethanol-DGAVP. BAC in samples taken after completion of the last trial were the same in all groups (sucrose-saline 283±6 mg %; sucrose-DGAVP 281±4 mg %; ethanol-saline 277±5 mg %; ethanol-DGAVP 284±4 mg %).

chronic DGAVP administration on the rate of 5-HT synthesis in the hippocampus and septum, in relation to the peptide-induced maintenance of ethanol tolerance. While several reports suggest that the central effects of vasopressin-like peptides are related to their ability to alter the turnover of catecholamines [10,20], to our knowledge, no studies have yet addressed the *in vivo* effects of these peptides on the turnover of indoleamines.

To study the *in vivo* rate of 5-HT synthesis, the accumulation of the serotonin precursor 5-HTP was measured after inhibition of the decarboxylation step by NSD 1015. As originally described by Carlsson *et al.* in 1972 [3], administration of NSD 1015 to rats resulted in the accumulation of 5-HTP in the brain. Under the present conditions, NSD 1015 promoted an accumulation of 5-HTP that was linear for up to 45 min in both the hippocampus and septum (data not shown). Rates of 5-HT synthesis calculated on the basis of the accumulation of 5-HTP at 30 min were comparable to those reported previously [3].

In agreement with the original report [3], NSD 1015 promoted no change in the concentrations of 5-HT and NA in the septum and hippocampus. This is likely the result of the ability of this compound to exert a simultaneous and complete inhibition of both the decarboxylation step leading to 5-HT synthesis and the oxidation of 5-HT catalyzed by MAO. This absence of appreciable changes in the steady state levels of 5-HT and NA is a condition necessary for a method aiming at measuring serotonin turnover rates. Finally, 5-HIAA levels were significantly decreased 30 min after the injection of NSD 1015, presumably as a result of a continued transport of brain 5-HIAA to the CSF, under conditions of an inhibited MAO.

To study the effects of *acute* DGAVP administration on 5-HT synthesis we initially chose a time period of 30 min between DGAVP injection and sacrifice (Experiment 1). This time period was selected on the basis of the observation that peak CSF concentrations of the parent peptide, AVP, had been reported 5 min after the SC administration of 5 μg AVP to rats, with CSF concentrations declining slowly over the next hour [14]. These investigators found that AVP was cleared from the CSF with a half-life of 26 min.

Thirty min, therefore, seemed a suitable period of time for DGAVP to reach its target tissue in the brain and possibly promote changes in serotonergic neurotransmission. Under

TABLE 3

EFFECT OF CHRONIC DGAVP TREATMENT ON THE RATE OF TRYPTOPHAN HYDROXYLATION AND MONOAMINE LEVELS IN THE HIPPOCAMPUS AND SEPTUM

Group	5-HTP (ng/g/30 min)	5-HT (ng/g)	5-HIAA (ng/g)	NA (ng/g)
A. Hippocampus				
Sucrose-saline	175 ± 11	439 ± 14	266 ± 11	1145 ± 54
Sucrose-DGAVP	188 ± 7	461 ± 21	297 ± 12	1154 ± 40
Ethanol-saline	170 ± 8	454 ± 23	289 ± 20	1085 ± 23
Ethanol-DGAVP	166 ± 14	454 ± 21	271 ± 13	1124 ± 35
B. Septum				
Sucrose-saline	277 ± 15	611 ± 29	297 ± 36	2367 ± 201
Sucrose-DGAVP	272 ± 16	559 ± 48	284 ± 27	2049 ± 135
Ethanol-saline	279 ± 18	592 ± 32	297 ± 26	1977 ± 74
Ethanol-DGAVP	248 ± 14	528 ± 51	239 ± 16*	2050 ± 127

Rats were sacrificed 30 min after the IP injection (200 mg/kg) of NSD 1015, and approximately 24 hr after the last SC injection of DGAVP (10 μg/rat) or saline. The hippocampi and septa were immediately dissected out, frozen on dry ice and kept at −70° C until assayed by HPLC-EC. n=10 per group. *Designates $p<0.05$ relative to ethanol-saline controls (Student's t-test for unpaired data).

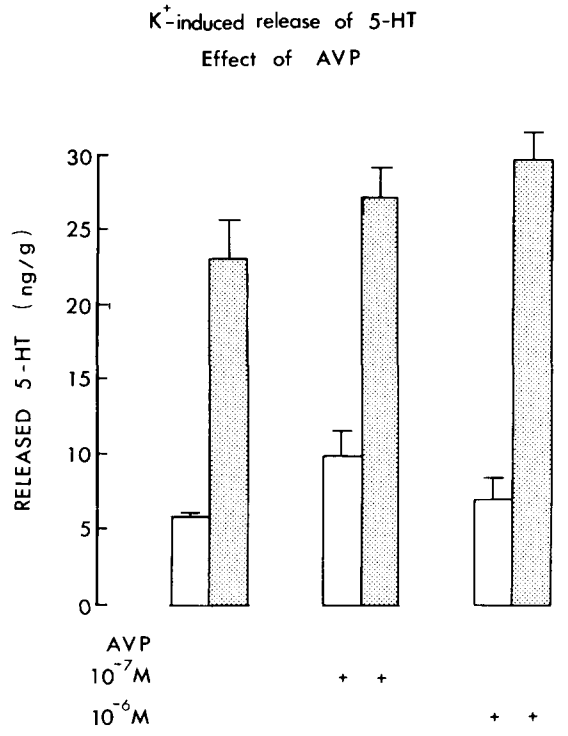

FIG. 3. Effect of AVP on the release of endogenous 5-HT. Hippocampal slices were incubated for 5 min in normal (open bars) or high K^+-KRP buffer (stippled bars), in the presence or absence of AVP. Bars represent group means±SEM (n=6).

these conditions, however, we failed to observe any effect of DGAVP on hippocampal or septal 5-HT synthesis. This was also the case in Experiment 2 (acute studies), where 4 hr were allowed between DGAVP injection and sacrifice. Changes in brain catecholamine turnover were reported to occur 4 hr after administration of lysine vasopressin to rats [19]. If VP-like peptides affect catecholaminergic and indoleaminergic neurons by similar mechanisms, then 4 hr should have sufficed for a DGAVP-induced alteration in the activity of 5-HT terminals.

It could also be argued the the lack of peptide effect results from the fact that in the acute studies, both the length of DGAVP treatment and the drug-related state of the animal subjects were markedly different from those generally used in experiments in which DGAVP maintains ethanol tolerance. Results from our chronic studies, however, do not support this argument. While chronic DGAVP administration to ethanol-tolerant subjects resulted in a long-term maintenance of central tolerance to ethanol-induced motor impairment, as previously reported [8, 11, 16, 18], the present study shows that this behavioral effect of DGAVP is not associated with changes in the rate of tryptophan hydroxylation in the hippocampus or septum.

These findings, therefore, do not support the concept of an *in vivo* presynaptic DGAVP-5-HT interaction leading to an enhanced neurotransmitter availability via increases in the 5-HT synthesis rate. Moreover, the observation that the synthesis of 5-HT was unaffected by the peptide treatment in conditions in which the levels of serotonin were also undisturbed by DGAVP, suggests that it is highly unlikely that peptide treatment had modified the *in vivo* release of 5-HT. In fact, had an increase in 5-HT release occurred without an increase in synthesis, it would have led to a sharp drop in the tissue concentration of 5-HT, and this did not occur.

In line with this argument, the *in vitro* studies indicate that addition of AVP (10^{-7} or 10^{-6} M) to the incubation medium failed to increase the potassium-induced release of endogenous 5-HT from naive hippocampal slices (Fig. 3). These results are in disagreement with those reported by Auerbach and Lipton [1] who found that AVP increased the potassium-stimulated release of 5-HT from hippocampal slices. The experimental conditions used in our studies were similar to those employed by Auerbach and Lipton [1], except for the strain of rats and the duration of the stimulation

period. While in our studies slices were subjected to a depolarizing medium for 5 min, Auerbach and Lipton stimulated for 10 min, which would permit higher chances for the occurrence of non-specific effects that could confound the experimental results. In this respect it is interesting to note that in the study by Auerbach and Lipton [1], incubation of slices in high-K^+ buffer resulted only in a two-fold increase in the amount of endogenous 5-HT released relative to the amount released under basal conditions. In our study, on the other hand, a 3- to 4-fold increase in 5-HT release was obtained, suggesting that our conditions provided a more sensitive estimation of the depolarization-induced release of endogenous serotonin. Whether these experimental differences could account for the discrepancy in results concerning the effects of AVP is unknown. The elucidation of this question would require a complete dose-response study with systematic variation of the duration of the incubation period and a comparison of results obtained with hippocampal slices from Wistar and Holtzman rats.

A lack of change in 5-HT release after incubation of hippocampal slices with AVP, however, is consistent with the observation that DGAVP fails to alter the synthesis or steady state levels of 5-HT in this brain region.

In our chronic studies, peptide-maintained tolerance to ethanol was not associated with changes in 5-HT synthesis or release just as tolerance produced by chronic ethanol administration was not accompanied by changes in 5-HT turnover rate [5].

This lack of effect of DGAVP treatment on the rate of 5-HTP accumulation in the hippocampus of tolerant rats, together with the inability of AVP to modify 5-HT release from the hippocampal slice, seems to argue against the hypothesis of a presynaptic DGAVP-5-HT interaction underlying the peptide effect on ethanol tolerance. It should be pointed out, nevertheless, that if the effects of DGAVP on alcohol tolerance involve an interaction with only a few 5-HT terminals, as suggested by our behavioral data [18], then it is conceivable that subtle peptide-induced changes in the 5-HT synthesis and/or release of some nerve terminals could have passed undetected in our measurement of total hippocampal 5-HT synthesis and release. A direct assessment of this possibility would require an evaluation of the rate of 5-HT synthesis or release in different regions of the hippocampus. In addition, the possibility remains open of a modulatory effect of DGAVP on the interaction of 5-HT with its postsynaptic target.

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