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Characterization of a uterine-type oxytocin receptor in the rat hippocampus

(electrophysiology/neurohypophyseal hormones/peptide analogues/structure–activity relationships)

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ABSTRACT **Extracellular recordings were obtained from a class of nonpyramidal neurones in hippocampal slices. Oxytocin applied to the bath at concentrations of 1 nM or greater excited these cells. This effect was reversibly antagonized by a synthetic structural analogue known to block the peripheral endocrine effects of oxytocin. The effect of oxytocin was mimicked by a selective oxytocic agonist and, with less potency, by vasopressin and by other structural analogues. The potencies of oxytocin, vasopressin, and of these analogues in the hippocampus correlated well with their uterotonic activities but not with their vasopressor or antidiuretic activities. The data suggest that a class of hippocampal neurones is endowed with receptors for oxytocin that are similar to those of uterine smooth muscle cells.**

Vasopressin and oxytocin are peptide hormones that are synthesized in neuronal cell bodies located in the hypothalamus and are carried by axoplasmic transport to the neurohypophysis, from which they are released into the general circulation in response to appropriate stimuli. In addition, they are also present in nerve fibers in various areas within the central nervous system (1). A calcium-dependent, depolarization-induced release of these peptides can be demonstrated from some areas of the brain where axons immunoreactive for vasopressin and oxytocin have been shown to terminate synaptically on neuronal elements (2, 3). This suggests that oxytocin and vasopressin, in addition to their general hormonal effects, might act as neurotransmitters or neuromodulators in the brain.

It has been shown that vasopressin and oxytocin increase the rate of firing of a class of neurones in the CA1 area of hippocampal slices. This electrophysiological response was not due to the peptides interacting with a receptor resembling the renal antidiuretic vasopressin receptor (4). However, at least two other types of endocrine receptors for neurohypophyseal hormones have been recognized. One is the vasopressor receptor, present on vascular smooth muscle cells and on liver cells, which triggers the vasopressor and glycogenolytic effects of vasopressin in these tissues (5–8). The other is an oxytocic receptor (9), which triggers the contractile response of the uterus to oxytocin. In this study, we attempted to assess whether receptors for oxytocin and vasopressin are similar in such widely different parts of the body as the brain and peripheral tissues responsive to these hormones. Because both vasopressin and oxytocin excited hippocampal neurones, it was possible that oxytocic receptors, vasopressor receptors, or a mixed novel class of receptors for neurohypophyseal peptides were involved. In theory, it should be possible to elucidate this point by using specific vasopressor antagonists and oxytocic antagonists. However, available antagonists are not truly specific and act on both vasopressor and oxytocic receptors (10). In contrast, highly selective oxytocic agonists are available (11). Therefore, we used a number of compounds possessing known endocrine effects (12) in an attempt to further characterize the nature of the hippocampal receptors. We now suggest that the receptors in the hippocampus for vasopressin and oxytocin have a greater similarity to oxytocin receptors responsible for smooth muscle contraction in the uterus than to those receptors mediating vasopressin effects.

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MATERIALS AND METHODS

Hippocampal Slices. Male Sprague–Dawley rats (about 250 g in body weight) were decapitated, and their brains were removed quickly. The left or right hippocampus was carefully dissected, and several 450-μm thick transverse slices were cut with a tissue chopper and transferred immediately to a thermoregulated (37°C) incubation chamber having a volume of 1 ml. The upper surfaces were exposed to a humidified gas mixture (95% O_2/5% CO_2), while their undersurfaces were supported on a nylon grid. The slices were perfused with 124.0 mM NaCl/5.0 KCl mM/26.0 mM $NaHCO_3$/1.4 mM $MgSO_4$/1.0 mM $CaCl_2$/1.24 mM KH_2PO_4/10.0 mM glucose (13).

Extracellular Recordings. Recordings from single neurones located in the pyramidal cell layer of the CA1 area of the hippocampus were obtained by using conventional techniques. Glass micropipettes filled with 4 M NaCl were used (tip diameter, 2–3 μm; tip resistance, 5–15 MΩ). Action potentials were displayed on an oscilloscope and stored on magnetic tape. A continuous rate-meter record of firing also was obtained "on-line" and plotted on a paper chart. Recent studies have shown that at least two different populations of neurones can be distinguished by electrophysiological criteria in the CA1 area: pyramidal neurones and nonpyramidal neurones (14–16). As far as extracellular recordings are concerned, these criteria include: spike duration, spontaneous firing rate and pattern, and response to stimulation of an afferent pathway. For the latter purpose, a bipolar stimulation electrode made of twisted nichrome wires, isolated except at the end, was positioned in stratum radiatum. Usual parameters of stimulation were 50- to 100-μA rectangular constant-current pulses, 0.1 ms in duration, 0.2–1.0 Hz.

Peptides. Arginine vasopressin (vasopressin with L-arginine in position 8) and oxytocin were produced in the Department of Biochemistry, Medical College of Ohio, Toledo, or purchased from Bachem, Bubendorf, Switzerland. A great number of structural analogues of the neurohypophyseal hormones also have been synthesized. These analogues possess widely differing agonistic or antagonistic effects, or both, on peripheral tissues responsive to oxytocin and vasopressin. Because no specific oxytocic antagonist is available, we compared the effect of oxytocin in the hippocampus with that of several agonists. These

compounds included the nonmammalian neurohypophyseal hormone arginine vasotocin (vasotocin with L-arginine in position 8), as well as four unnatural analogues, all synthesized at Toledo: [2-phenylalanine, 8-ornithine]vasotocin; 1-deamino-[4-valine, 8-D-arginine]vasopressin; [1-(L-α-hydroxy-β-mercaptopropionic acid), 4-threonine, 7-glycine]oxytocin; 1-deamino-[2-(O-methyltyrosine), 4-valine, 8-D-arginine]vasopressin. The biological activities of all peptides used are given in Table 1. They were dissolved in medium immediately before use, and the slices were perfused at a rate of 2.0 ml/min, the dead space of the inflow tubing leading to the chamber being approximately 2.0 ml.

RESULTS

Effects of Oxytocin and Vasopressin. The majority of cells in the CA1 area displayed no spontaneous activity or fired at a low average rate (0.5–5.0 spikes per s), with action potentials occurring singly and only occasionally in doublets. Stimulation of stratum radiatum usually evoked only a single action potential from either of these cells. At a higher stimulus intensity, the latency of this action potential was seen to be the same as that of the population spike. Therefore, these cells were deemed to be pyramidal neurones. Another class of cells displayed smaller and shorter action potentials and fired spontaneously at a higher mean rate (10–30 spikes per s), often in short intermittent trains. These are characteristics attributed to nonpyramidal neurones (16).

Long-term extracellular recordings (1–6 hr) were obtained from over 200 presumptive nonpyramidal neurones. Vasopressin or oxytocin excited more than 90% of these cells and inhibited none. As expected, stimulation of the alveus did not trigger an antidromic action potential from such cells (14). In a few cases, they were further characterized by their response to an orthodromic input (14, 16): stratum radiatum stimulation evoked a burst of action potentials that outlasted the duration of the population spike; the first spike of the burst could occur before the onset of the population spike. All 10 cells so identified were excited by vasopressin or oxytocin (Fig. 1).

In the preliminary studies reported in a previous article, we noticed that oxytocin exerted similar effects and was at least as powerful as arginine vasopressin (4). Therefore, we endeavored to compare systematically the effects of oxytocin and vasopressin by establishing dose–response curves. Each cell recorded from was exposed to vasopressin at 1 μM and to at least two further concentrations of vasopressin or oxytocin applied in random order. We computed in each instance the increase in firing rate from the resting level to the peak level reached in the presence of the peptide at each concentration. Fig. 2 shows these results for peptide concentrations ranging from 0.1 nM to 10 μM. The lowest effective concentration for oxytocin that was tested was 1 nM, and stimulation was maximal at approximately 1 μM, the effect being half maximal at around 10 nM. Although for vasopressin a plateau was not obtained even at the highest concentration tested (10 μM), the two curves were found to run parallel, vasopressin producing a much smaller increase in firing than oxytocin when applied at the same concentration. Because this greater sensitivity to oxytocin was noticed for every cell tested, we may assume that oxytocin and vasopressin acted on a homogenous population of cells.

Effects of Structural Analogues. The respective oxytocic, antidiuretic, and vasopressor activies of all peptides tested are given in Table 1, ranked according to increasing oxytocic activities. Compound I is thus devoid of oxytocic agonist activity (it is an oxytocic antagonist), whereas compound VII was the most powerful and specific oxytocic agonist available. The potency of each compound in the hippocampus was determined by applying it at 1 μM for 5 min and by counting the total number of action potentials generated in response to peptide application. To facilitate comparison, the potency of each compound was expressed relative to that of vasopressin applied at the same concentration in every experiment. The results, shown in Figs. 3 and 4, relate the relative potency of each compound with its endocrine activities (on a log scale) taken from Table 1. It is apparent that the relative potency of all seven compounds correlates well with their oxytocic activity but bears no direct relationship with either their vasopressor or antidiuretic activities (Fig. 4).

The oxytocin analogue [1-(L-α-hydroxy-β-mercaptopropionic acid), 4-threonine, 7-glycine]oxytocin (compound VII), oxytocin, and arginine vasotocin possessed similar potencies when applied at 1 μM, the response being probably close to maximum (Fig. 3). At 10 nM (five cells in four slices) the oxytocin analogue was found to be twice as potent and arginine vasotocin to be 1/3 less potent than oxytocin (data not shown), again in good agreement with their respective oxytocic activities (see Table 1).

Of the analogues tested, 1-deamino-[2-(*O*-methyltyrosine), 4-valine, 8-*D*-arginine]vasopressin (compound I) was the only one that acted like an oxytocic antagonist on uterine smooth muscle. Applied at 0.1 μM, it reduced markedly but not totally the excitatory effect of oxytocin at 0.1 μM on nonpyramidal neurones. At 1 μM, it totally and reversibly antagonized the effect of oxytocin at 0.1 μM, in five out of five cells tested (Fig. 5).

Table 1. Endocrine activities of oxytocin, arginine vasopressin, and analogues

	Peptide[a]	Endocrine activities, units/mg: Oxytocic[b]	Antidiuretic[c]	Vasopressor[d]
I	d[Tyr(Me)^2,Val^4, D-Arg^8]VP[e]	0[f]	≈2,000	0[f]
II	d[Val^4,D-Arg^8]VP[g]	2	1,230	0[f]
III	[Phe^2,Orn^8]VT[h]	5	1.6	121
IV	[L-Arg^8]VP	26	323	369
V	[L-Arg^8]VT	246	295	227
VI	OT	486	4	4
VII	HO[Thr^4,Gly^7]OT[i]	1,002	0.004	<0.01

[a] VP, vasopressin; VT, vasotocin; OT, oxytocin; [L-Arg^8]VP, arginine vasopressin; [L-Arg^8]VT, arginine vasotocin. Amino-acid substitutions are indicated by the usual abbreviations. Unnatural amino acids: Orn, ornithine, Tyr(Me), *O*-methyltyrosine. Abbreviations for replacement of 1-cysteine in compounds I, II, and VII: d, substitution of 1-β-mercaptopropionic acid (i.e., deamino derivative of 1-cysteine); HO, substitution of 1-(L-α-hydroxy-β-mercaptopropionic acid).

[b] Determined *in vitro* on rat uterine horns in medium containing 0.5 mM Mg.

[c] Determined in rats under ethanol anesthesia.

[d] Determined in phenoxybenzamine-treated rats.

[e] Unpublished data.

[f] Antagonist.

[g] Sawyer *et al.* (17) and Manning *et al.* (18).

[h] Berde *et al.* (19). Activities are from assays on a new synthesis of this peptide (unpublished data).

[i] Lowbridge *et al.* (11).

DISCUSSION

The data are consistent with our suggestion that an excitatory effect of oxytocin and vasopressin in the hippocampus might result from an interaction with central receptors similar to the oxytocic receptors present in the uterus. This conclusion was derived from recordings obtained from a class of nonpyramidal neurones that discharged spontaneously in the slices and re-

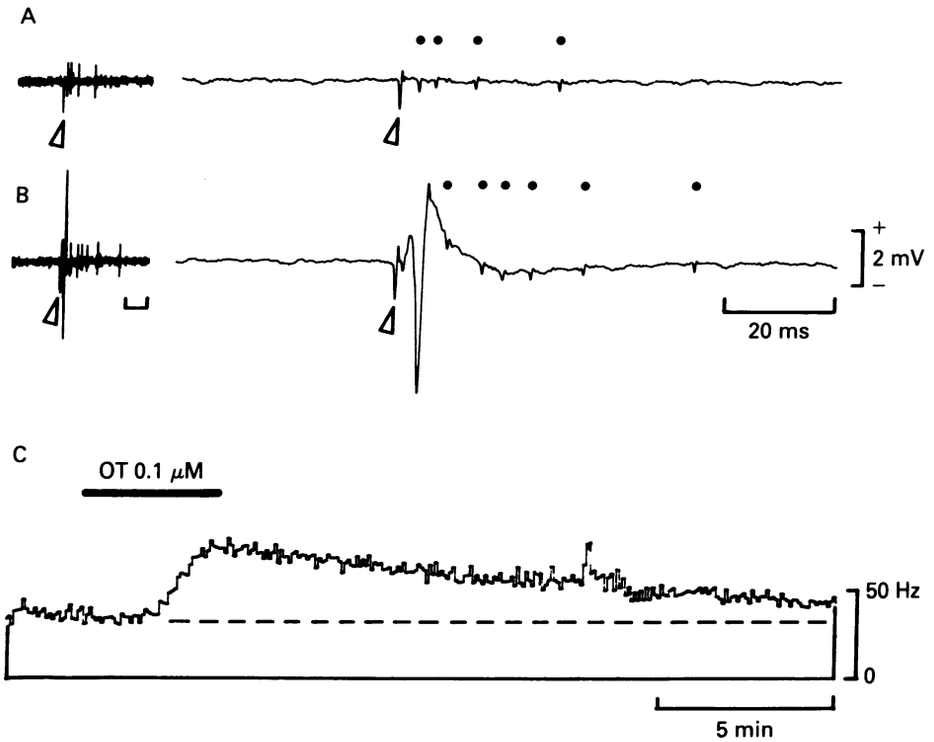

FIG. 1. Characterization of a hippocampal neurone as being nonpyramidal (*A* and *B*) and its response to oxytocin (*C*). *A* and *B* illustrate the response of this cell to stimulation of stratum radiatum at two intensities, one subthreshold (*A*) and the other above threshold (*B*) for generation of a population response. In *A* and *B*, the same response is shown twice: at low speed and under conditions of DC recording (*Right*) and 5 times faster and filtered (bandwidth, 0.1–3.0 kHz) (*Left*). Stimulation artefacts are indicated by open arrowheads. Note that stimuli trigger a burst of action potentials marked by black dots (*Right*), even when subthreshold for elicitation of a population response (*A Right*). The bursts outlast the duration of the fast, negative component of the population response (*B Right*), which corresponds to the synchronized discharge of pyramidal neurones. In *B*, note that the early negative response after the stimulus artefact might contain an early driven action potential, although difficult in this instance to distinguish from the input volley. (*C*) Rate-meter record shows this spontaneously active neurone to be excited by oxytocin (OT) at 0.1 μM applied during the period indicated by the thick horizontal line.

sponded to the bath application of oxytocin, vasopressin, and some of their structural analogues by a reversible increase in firing rate. The response to a chosen concentration of peptide served as the basis of an "electrophysiological assay" that allows an indirect characterization of the receptors involved. Other, indirect, means of receptor characterization have been used previously to study the endocrine receptors for oxytocin and vasopressin. These methods include assessing the effects of neurohypophyseal hormones (*i*) on membrane phospholipid turnover (6–8), (*ii*) on the level of intracellular second messengers (5), and (*iii*) in a variety of assays performed *in vivo* or

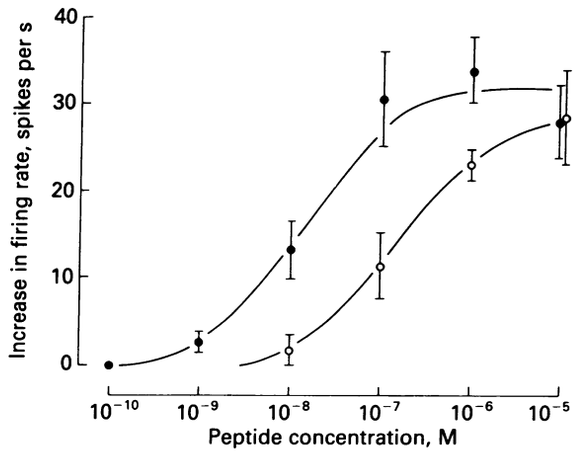

FIG. 2. Effects of oxytocin (●) and arginine vasopressin (○) applied at various concentrations on the firing of nonpyramidal neurones. Each point shows the mean increase [± SEM (n = 5) except for oxytocin at 1 μM, where n = 13, and arginine vasopressin at 1 μM, where n = 23] in firing rate at the concentration of peptide indicated. Oxytocin at 0.1 nM never had an effect; consequently, this point has no SEM.

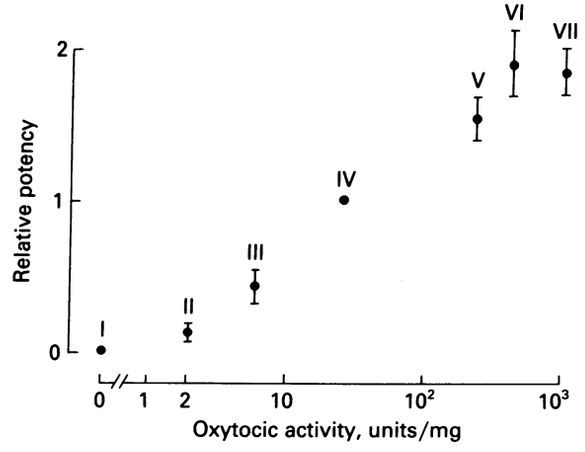

FIG. 3. Relative potency in the hippocampus versus their oxytocic activities of the seven compounds of Table 1. Each point shows the mean increase in firing rate induced by the compounds relative to the increase induced by arginine vasopressin, all applied at 1 μM. SEMs are given (with $n \geq 5$) except for compound IV (arginine vasopressin, serving as internal standard; n = 29) and compound I (which was inactive; n = 8). Note that relative potency increases with increasing oxytocic activity.

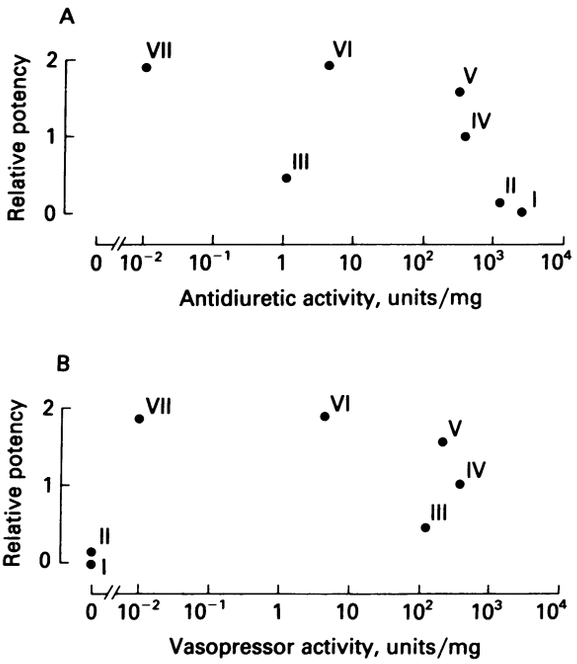

FIG. 4. Relative potency versus antidiuretic (*A*) or vasopressor (*B*) activities described in Table 1 (second and third columns). SEMs, being the same as in Fig. 2, have been omitted for clarity. Note the lack of correlation between the potency of these seven compounds in hippocampus and their activity as antidiuretic or vasopressor agonists (e.g., compound VII, which virtually lacks any vasopressor or antidiuretic activity, is an extremely potent agonist in the hippocampus).

using isolated organs (12, 20). The assay used in the present study is comparable to these other, indirect, means of receptor characterization.

That the hippocampal response that we studied is due to an interaction with oxytocic receptors is supported by several observations. First, oxytocin was found to be much more potent than vasopressin. Table 1 shows that in the isolated uterus, vasopressin produces a contraction (26 units/mg) equivalent to that produced by 1/18.7 times as much oxytocin (486 units/mg). The shift of the dose–response curves in the hippocampus (Fig. 2) yields a similar value. Second, for a variety of structural analogues, the relative potency of each compound correlated well with its activity as an oxytocic agonist in the isolated uterus (Fig. 3). In contrast, no correlation was obtained with either antidiuretic or vasopressor activities (Fig. 4). Third, amongst these analogues, a *selective* oxytocic agonist (compound VII) had a powerful effect in the hippocampus, whereas an analogue acting as an antagonist in the uterus (compound I), blocked the response to oxytocin (Fig. 5). Finally, the sensitivity of the hippocampus to oxytocin does not differ widely from that of the uterus. In the isolated rat uterus, the half-maximal response to oxytocin necessitates 1–2 nM, and this value agrees closely with that obtained for the dissociation constant (K_d) of tritiated oxytocin bound to uterine membranes (9). In the hippocampus, we estimate the K_d to be around 10×10^{-9} M (Fig. 1). However, some peptide degradation may occur in the slices, and the actual K_d at the receptor level may be even closer to that found for the uterus.

Our preliminary studies had shown that both oxytocin and vasopressin excite hippocampal neurones (4); however, we could not tell whether they act on vasopressor, oxytocic, or mixed (vasotocin) receptors. The antagonist used in the early experiments, [1-(β-mercapto-β,β-cyclopentamethylene-propionic acid), 2-(*O*-methyltyrosine), 8-L-arginine]vasopressin, lacks selectivity in this respect; it is a strong antivasopressor compound, but it also displays considerable antioxytocic activity (10). In view of the present results, it is probable that this compound blocked the effect of vasopressin in the hippocampus by an action on oxytocic receptors. This conclusion is reinforced by the observation that this compound reduced only very slightly the effect of oxytocin applied at the same concentration (unpublished observations).

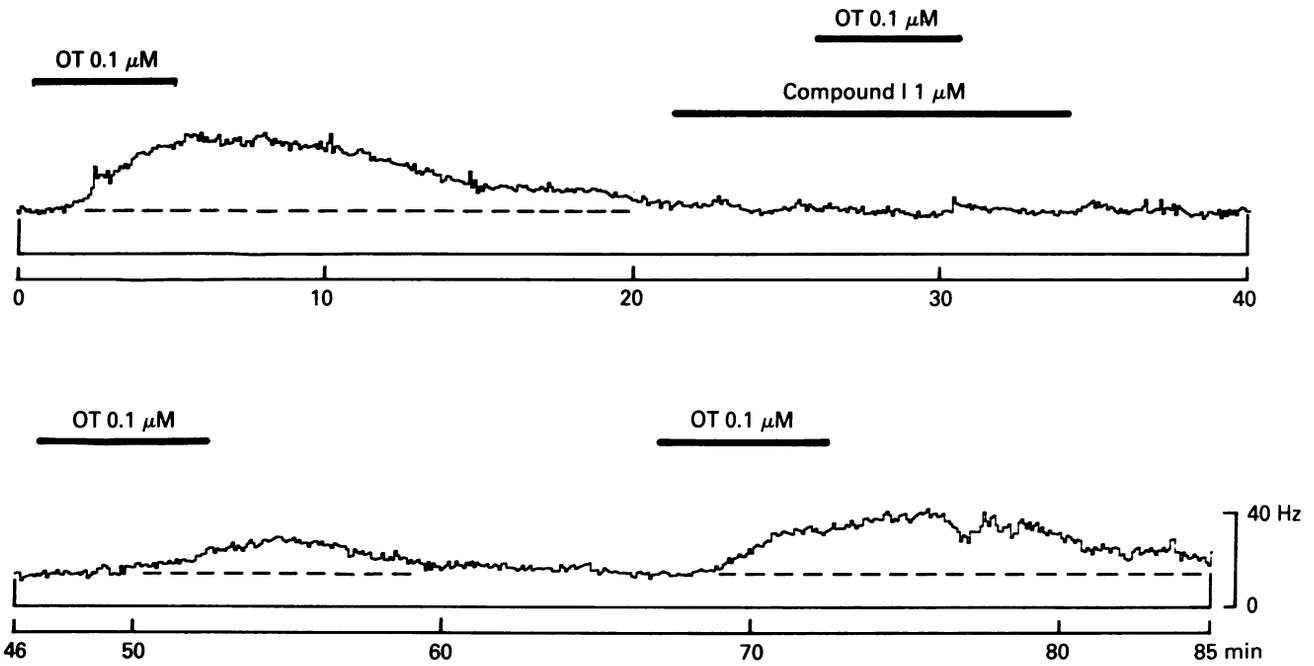

FIG. 5. Reversible antagonism of oxytocin (OT) effect on a CA1 nonpyramidal neuron by a synthetic structural analogue which is an oxytocic and vasopressor antagonist. OT at 0.1 μM was applied to the bath (during the periods indicated by thick lines) before, during, and after application of the antagonist 1-deamino-[2-(*O*-methyltyrosine), 4-valine, 8-D-arginine]vasopressin (compound I) at 1 μM. *Upper* and *Lower* constitute a continuous rate-meter record except for a 6-min deletion.

Although the identification of the primary target of neurohypophyseal peptides in the hippocampus is not the aim of this report, the following observations lead to the conjecture that oxytocin and vasopressin directly excite a class of nonpyramidal neurones and that these could in turn inhibit pyramidal neurones. (*i*) Virtually all cells that fulfilled the criteria held to be typical of nonpyramidal neurones were excited. (*ii*) This effect persisted after synaptic uncoupling (4). (*iii*) Pyramidal neurones were not excited but either unaffected or inhibited, as evidenced by a membrane hyperpolarization or a reduction in firing rate (or both) in those that fired spontaneously (21). (*iv*) This inhibition of pyramidal neurones was possibly caused by an indirect process because neurohypophyseal peptides also increased the rate of occurrence of spontaneous inhibitory postsynaptic potentials in these cells (21).

In the hippocampus, there are fewer interneurones than pyramidal neurones, but the axons of the former have extensive local ramifications (14, 22). The presence in the hippocampus of vasopressin-containing fibers but not that of oxytocin-containing fibers had been reported. Recently, however, Sofroniew found that similar patterns of fibers stained positively not only for vasopressin but also for oxytocin (23). Although the density of fibers that are immunoreactive to vasopressin or oxytocin is moderate, the interneurones responsive to neurohypophyseal peptides could modulate transynaptically the activity of a great number of pyramidal neurones. It is generally accepted that a similar mechanism explains the widespread effects of opiate-like peptides in the hippocampus. Enkephalins have been shown to excite pyramidal neurones by an indirect process, i.e., by inhibiting a class of inhibitory interneurones (24).

Several studies suggest that centrally acting neurohypophyseal hormones can induce or modulate various behaviors. Vasopressin has received much attention and may be involved in tasks in which past experience plays a role (25, 26). According to structure–activity data, these effects could be mediated by an interaction with receptors different from the known vasopressin receptors (27). In a recent study, however, Abe *et al.* (28) have studied the effects of vasopressin and oxytocin in hypothalamic slices; they found that both peptides could depolarize supraoptic neurones by acting on renal-type vasopressin receptors. It remains to be seen if other vasopressin receptors in the brain belong to the same class.

Oxytocin is also capable of exerting powerful central actions. Thus, after intracerebroventricular injection, it induces maternal behavior in virgin female rats (29, 30) and facilitates the milk-ejection reflex in lactating rats (31). These two central effects of oxytocin are functionally related to the endocrine actions exerted by oxytocin on the uterus and the mammary gland. Further studies are required to elucidate the function of oxytocin and vasopressin in the hippocampus.

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