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Correlation between oxytocin neuronal sensitivity and oxytocin receptor binding: An electrophysiological and autoradiographical study comparing rat and guinea pig hippocampus

(brain slices/CA1 field/cholinomimetics/interneurones/ventral subiculum)

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ABSTRACT **In transverse hippocampal slices from rat and guinea pig brains, we obtained unitary extracellular recordings from nonpyramidal neurones located in or near the stratum pyramidale in the CA1 field and in the transition region between the CA1 and the subiculum. In rats, these neurones responded to oxytocin at 50–1000 nM by a reversible increase in firing rate. The oxytocin-induced excitation was suppressed by a synthetic structural analogue that acts as a potent, selective antioxytocic on peripheral receptors. Nonpyramidal neurones were also excited by carbachol at 0.5–10 μM. The effect of this compound was postsynaptic and was blocked by the muscarinic antagonist atropine. In guinea pigs, by contrast, nonpyramidal neurones were unaffected by oxytocin, although they were excited by carbachol. Light microscopic autoradiography, carried out using a radioiodinated selective antioxytocic as a ligand, revealed labeling in the subiculum and in the CA1 area of the hippocampus of rats, whereas no oxytocin-binding sites were detected in the hippocampus of guinea pigs. Our results indicate (*i*) that a hippocampal action of oxytocin is species-dependent and (*ii*) that a positive correlation exists between neuronal responsiveness to oxytocin and the presence in the hippocampus of high-affinity binding sites for this peptide.**

The nonapeptide oxytocin, which was originally characterized—with vasopressin—as one of the hypothalamo-neurohypophysial hormones, probably also functions as a neurotransmitter in selected areas of the mammalian central nervous system (1, 2). Electrophysiological evidence supporting a central effect of oxytocin includes the finding that oxytocin directly excites nonpyramidal neurones in hippocampal slices from rats (3), probably by acting on oxytocin receptors similar to those present in the uterus (4). In addition, dorsal vagal motoneurones in brainstem slices from rats are the target of a stimulatory action of this peptide (5).

High-affinity binding sites for [^{3}H]oxytocin have been detected in the rat brain by light microscopic autoradiography (6, 7). These findings are in accordance with the notion that oxytocin may act as a neurotransmitter. However, alternative interpretations of autoradiographic studies cannot be excluded. Indeed, binding sites may be located on nonneuronal cells, such as glial or endothelial cells; binding sites may not correspond to functional receptors or may represent receptors whose activation initiates metabolic or structural changes in the neurone rather than generating bioelectrical signals. One aim of the present work was thus to ascertain whether oxytocin binding sites represent functional neuronal receptors.

In a recent study, we found that oxytocin depolarizes virtually every vagal neurone in the rat brainstem, whereas it was without effect on vagal neurones in the guinea pig (8). However, no correlation between responsiveness to oxytocin and the existence of oxytocin binding sites in the dorsal motor nucleus of the vagus nerve could be established at the time of that study, probably as a result of the low specific activity of the [^{3}H]oxytocin used for autoradiography (see ref. 6).

In the present work, we have used electrophysiological techniques and hippocampal slices from the rat and the guinea pig to assess whether in another area of the brain the action of oxytocin—and, for comparison, that of a cholinomimetic compound, carbachol [(2-hydroxyethyl)trimethylammonium chloride carbamate]—is also species-dependent. In addition, by making use of autoradiography, and of a recently available selective, radioiodinated ligand of the oxytocin receptor, we have asked whether a positive correlation exists between the neuronal sensitivity to oxytocin and the presence in the hippocampus of high-affinity oxytocin binding sites.

MATERIALS AND METHODS

Hippocampal Slices. Experiments were done using male adult rats (150–250 g) from the Sivz strain—a Sprague–Dawley-derived strain—and male adult guinea pigs from the Dunkin–Hartley strain (250–400 g). Following decapitation, the brain was removed, one hippocampus was dissected, and, from its ventral region, four or five transverse slices (300–400 μm thick) were cut. The slices were laid down on a nylon grid, in a thermoregulated (35–36°C) recording chamber, at the interface between an oxygenated, humidified atmosphere and a perifusion solution, which contained (in mM) NaCl, 135; KCl, 5; $NaHCO_3$, 15; $MgSO_4$, 1; KH_2PO_4, 1.2; glucose, 10; and $CaCl_2$, 1. The perifusion solution was saturated with 95% O_2/5% CO_2, had a pH of 7.3–7.4, and flowed at 2–3 ml/min. The time needed to completely exchange the content of the recording chamber was 2–3 min. Recordings were usually started 1–2 hr after the preparation of the slices. Synaptic uncoupling was achieved by perifusing the preparation with a solution that contained 0.1 mM $CaCl_2$ and 4 mM $MgSO_4$, instead of the usual concentration of divalent cations.

Electrophysiological Recordings. In both species, recordings were obtained from neurones located in or near the

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Abbreviations: atropine, atropine sulfate monohydrate; carbachol, (2-hydroxyethyl)trimethylammonium chloride carbamate; des-Gly(NH_2)-d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine,8-ornithine,9-desglycinamide]vasotocin; d(CH_2)$_5$[Tyr(Me)^2, Thr^4,Tyr(NH_2)^9]OVT, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine,8-ornithine,9-tyrosinamide]vasotocin.

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stratum pyramidale in field CA1 and in the CA1/subiculum transition region. Single-unit activity and field potentials were recorded extracellularly, using glass micropipettes filled with 4 M NaCl (tip resistances, 5–20 MΩ). When intracellular recordings were carried out, the $CaCl_2$ concentration was raised to 2 mM. Glass micropipettes, filled with 3 M potassium acetate (pH 7.4; tip resistances, 40–120 MΩ), were used. Amplified signals were displayed on an oscilloscope either under ac (band width, 0.1–3.0 kHz) or dc conditions. Ratemeter records of single-unit firing and membrane potential were monitored on paper. Orthodromic activation of hippocampal neurones was achieved by electrical stimulation of Schaffer's collaterals. Bipolar stimulation electrodes, made of twisted nichrome wires (100 μm in diameter, isolated except for their tips), were positioned in the stratum radiatum. Stimuli were constant-current pulses (25–350 μA, 0.1 ms, 0.4–1 Hz).

Light Microscopic Autoradiography. The animals were decapitated and the brains were removed and frozen in 2-methylbutane at −25°C. Sections, 15 μm thick, were cut, thaw mounted on gelatin-coated slices, dehydrated under vacuum, and stored at −80°C until use. For the binding procedure, sections were lightly fixed by dipping the slides in a solution of 0.2% paraformaldehyde in phosphate-buffered saline (pH 7.4) and then rinsed twice for 5 and 10 min in 50 mM Tris·HCl (pH 7.4). Incubation was carried out for 1 hr at room temperature in a humid chamber by covering each slide with 400 μl of the incubation medium (50 mM Tris·HCl/0.1 mM bacitracin/5 mM $MgCl_2$/0.1% bovine serum albumin) containing 0.05 nM ^{125}I-labeled [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine,8-ornithine,9-tyrosinamide]vasotocin {^{125}I-d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Tyr$(NH_2)^9$]OVT}.

Nonspecific binding was determined by incubating adjacent sections in the same medium containing, in addition, 100 nM oxytocin. Incubation was followed by two 5-min washes in ice-cold incubation medium and a quick rinse in distilled water. The slides were rapidly dried and apposed to Kodak X-OMat AR film for 5 days. Films were developed for 5 min in Kodak D19 and the sections were stained with cresyl violet.

Chemicals. Oxytocin was obtained from Bachem and from Nova Biochem (Läufelfingen, Switzerland). Carbachol and atropine (atropine sulfate monohydrate) were from Fluka; oxotremorine {1-[4-(pyrrolidinyl)-2-butynyl]-2-pyrrolidinone} was from Sigma. The oxytocin antagonist [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-*O*-methyltyrosine,4-threonine,8-ornithine,9-desglycinamide]vasotocin {des-Gly(NH_2)-d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT} (see ref. 9) was a gift from M. Manning (Toledo, OH) and W. H. Sawyer (New York). All of these compounds were tested by dissolving them in the perifusion medium. The radioiodination of d$(CH_2)_5$[Tyr(Me)^2,Thr^4,Tyr$(NH_2)^9$]OVT (see ref. 9) was performed by C. Barberis and S. Jard, at Montpellier, France (10).

RESULTS

Electrophysiological Studies in Slices from Rats. Single-unit extracellular recordings were obtained from 25 neurones in 23 hippocampal slices from rats. The recorded cells were all spontaneously active, discharging at rates ranging from 2 to 20 spikes per s. When activated orthodromically they responded by a train of action potentials, which started before the onset of the pyramidal population spike and which outlasted it; they were thus presumed to be nonpyramidal

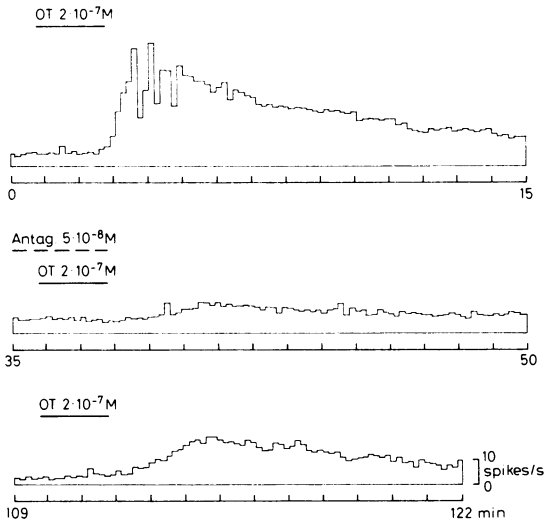

FIG. 1. Effect of oxytocin (OT) and its antagonism by des-Gly(NH_2)-d$(CH_2)_5$[Tyr(Me)^2,Thr^4]OVT (Antag.) on a nonpyramidal hippocampal neurone from the rat. Each trace represents the neuronal rate of discharge (ordinate) as a function of time (abscissa). Compounds were added to the perifusion solution at the concentration indicated and for the time corresponding to the solid line above each trace. The antagonist, added at the 24th minute, did not have any effect *per se* but almost totally suppressed the excitation induced by oxytocin (second trace). The effect of oxytocin partially recovered following extensive wash out (third trace). The traces are consecutive but not continuous.

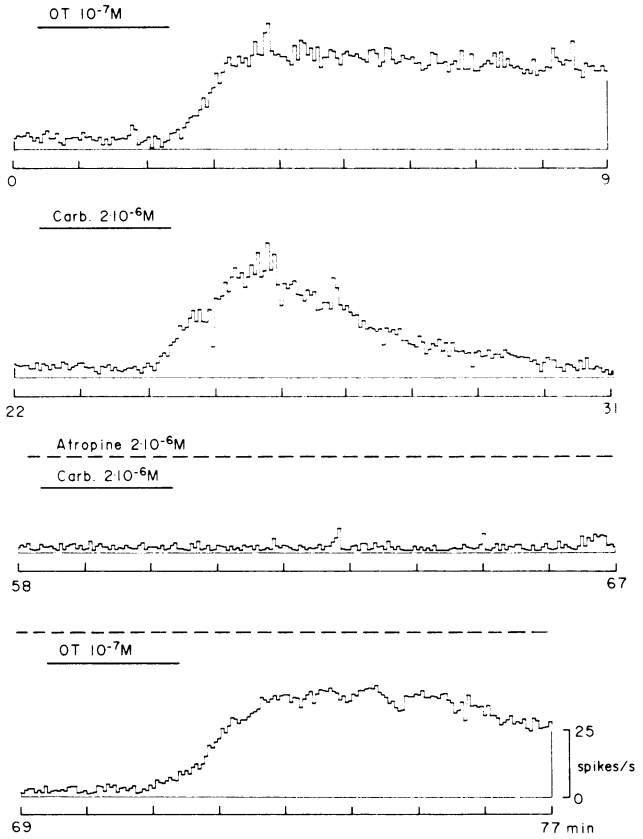

FIG. 2. Effect of oxytocin (OT), carbachol (Carb.), and atropine on a nonpyramidal hippocampal neurone from the rat. Oxytocin and carbachol were added at the concentration indicated. Atropine, 2 μM, was present from the 53rd minute onward (broken line). Atropine did not affect the spontaneous firing rate of the neurone; it suppressed the excitation brought about by carbachol (third trace) but not that caused by oxytocin (fourth trace). Note that, at the concentrations used, the effect of carbachol (second trace) was readily reversible, whereas that of oxytocin, although also reversible, was more sustained (first and fourth traces). The traces are consecutive but not continuous.

cells (4). As previously reported, oxytocin, added to the perifusion solution at 50–1000 nM, caused a reversible excitation of all of these neurones (see Figs. 1 and 2, first trace). The synthetic structural analogue des-Gly(NH_2)-d(CH_2)$_5$[Tyr(Me)^2,Thr^4]OVT, added at 50–1000 nM, partially or completely suppressed the effect of 50–500 nM oxytocin (tested on 7 neurones). At the lowest concentration of analogue (50 nM), antagonism was incomplete and partially reversible (Fig. 1); at higher concentrations (100–1000 nM), antagonism was full but almost irreversible.

Carbachol, a cholinomimetic compound, excited 14 of 14 nonpyramidal neurones. At 0.5 μM, it caused an increase in the rate of discharge, above the resting level, of 12 ± 3 spikes per s (mean ± SEM; n = 5); at 2 μM, firing increased by 29 ± 5 spikes per s (n = 8; Fig. 2, second trace). At both concentrations the carbachol-induced excitation was readily reversible. At 10 μM, the effect of carbachol, although still reversible, lasted much longer; in 2 neurones the induced increase in the rate of discharge was 40 and 55 spikes per s, respectively, and in 2 other neurones a paroxystic excitation occurred, during which bursts of action potentials—with intraburst frequencies higher than 70 spikes/s—alternated with pauses in firing. In all 4 cells tested the action of carbachol at 2 μM was blocked by the muscarinic antagonist atropine, added at 2 μM (Fig. 2, third trace); atropine was without effect on the oxytocin-induced excitation (Fig. 2, fourth trace). Carbachol acted—at least in part—postsynaptically since, at 1–2 μM, its excitatory effect persisted when the preparation was perifused with a low-calcium, high-magnesium solution in which synaptic transmission was suppressed (Fig. 3, tested on 6 neurones).

The selective muscarinic agonist oxotremorine (11) excited 4 of 4 nonpyramidal neurones; at 1 μM it brought about an increase in firing of 18 ± 4 spikes per s. This action was long-lasting and only partially reversible.

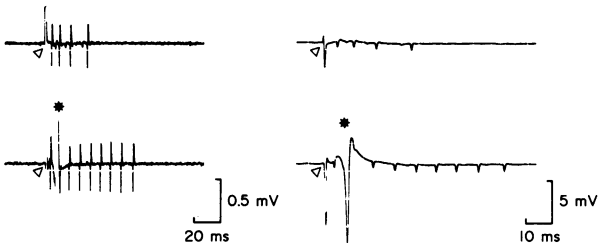

FIG. 4. Response of a guinea pig hippocampal neurone to orthodromic activation. Stimulus strength was either subthreshold (upper traces) or above threshold (lower traces) for evoking a pyramidal cell population spike. Left panels show filtered ac oscilloscope traces; right panels show the same traces recorded under dc conditions and at two times faster sweep speed. Positivity is upward; open triangles mark stimulus artefacts. A weak stimulus triggered a burst of four and a stronger stimulus triggered a burst of eight action potentials from the recorded neurone. Note that the bursts started before and outlasted the pyramidal cell population spike (asterisk). The responses of this neurone to oxytocin and carbachol are shown in Fig. 5*B*.

Electrophysiological Studies in Slices from Guinea Pigs. Extracellular recordings were obtained from 18 neurones in 15 guinea pig hippocampal slices. Four neurones were silent, whereas the remaining fired at average rates ranging from 2 to 35 spikes per s. Since they all responded to orthodromic activation by discharging repetitively (Fig. 4), they were deemed to be nonpyramidal neurones (12). Their chemosensitivity differed from that found in the rat: none of the guinea pig neurones responded to oxytocin at 1 μM, even though 11 of 11 were readily excited by carbachol at 1–10 μM (Fig. 5). This effect of carbachol was antagonized by atropine at 1 μM (tested on 4 neurones).

Intracellular recordings were obtained from 14 pyramidal cells in nine slices. Most were silent; some fired at frequen-

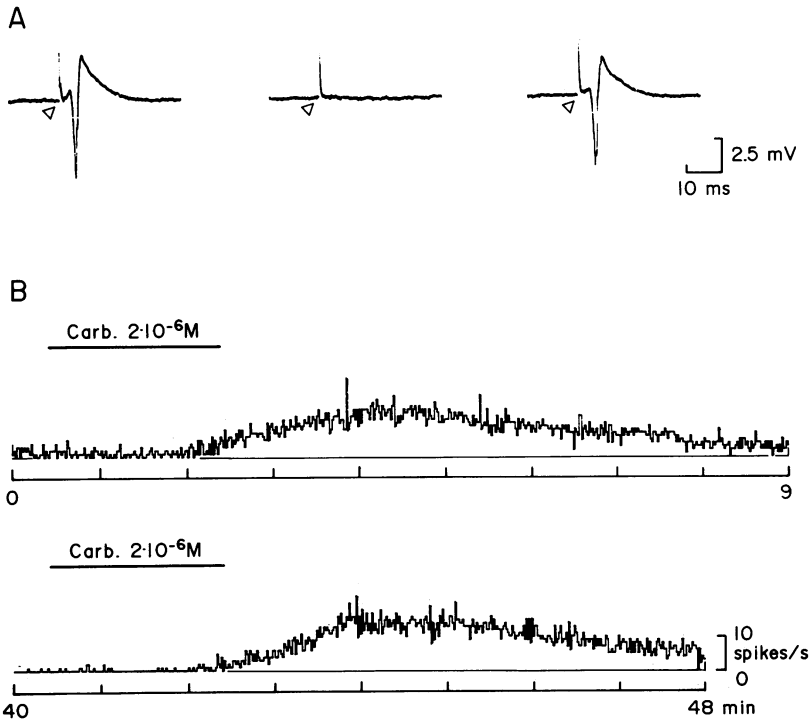

FIG. 3. Effect of carbachol (Carb.) on a rat nonpyramidal neurone in normal and in low-calcium, high-magnesium solution. (*A*) Evoked field potential recorded in the CA1 region in normal medium (left), after 8 min of perifusion with the modified solution (center), and 15 min following reintroduction of the normal solution (right). Positivity is upward. Stimulus artefacts are marked by open triangles. (*B*) Carbachol-induced excitation in normal solution (upper histogram) and after 8 min of perifusion with low-calcium, high-magnesium solution (lower histogram). Note that in the modified solution the evoked field potential reversibly disappeared and the neurone became almost silent; carbachol, however, fully retained its excitatory effect.

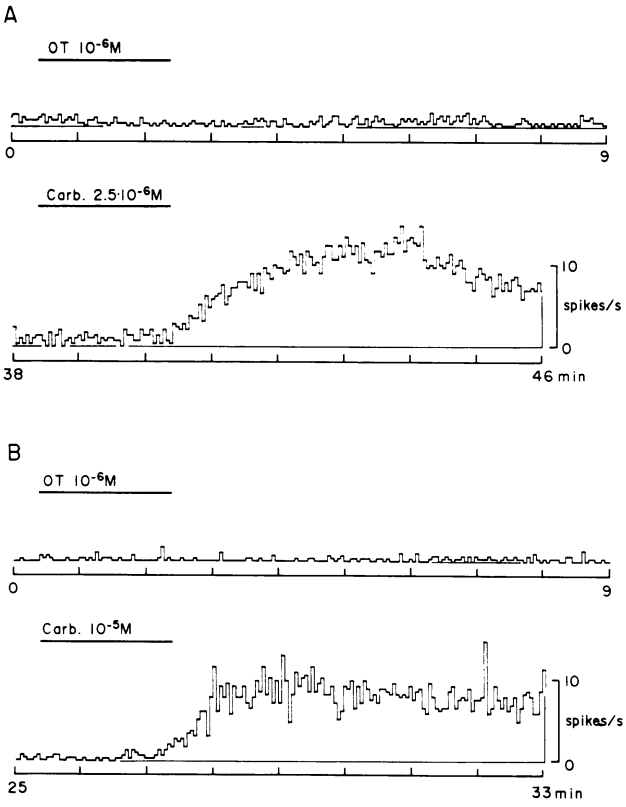

FIG. 5. (*A* and *B*) Effect of oxytocin (OT) and carbachol (Carb.) on two nonpyramidal guinea pig neurones. Note that oxytocin was without effect, whereas carbachol evoked a reversible increase in firing rate. The response of the neurone in *B* to orthodromic activation is illustrated in Fig. 4.

cies below 1 spike per s. Their average resting membrane potential was -61 ± 2 mV and their mean input resistance was 43 ± 4 MΩ. Oxytocin at 1 μM affected neither the frequency of discharge nor the resting potential (tested on 14 neurones) nor the input resistance (tested on 10 neurones).

Autoradiography. The distribution of specific high-affinity binding sites for oxytocin in the rat and guinea pig hippocampus was compared using a radioiodinated selective oxytocin antagonist as a ligand. Fig. 6*B* shows results obtained in the rat: the subiculum was intensely labeled; moderate to light labeling was present in the field CA1 pyramidal cell layer and in the CA1/subiculum transition region; moderate labeling was also observed in the entorhinal cortex and in the caudate putamen. In the guinea pig, oxytocin binding sites could not be detected in any subdivision of the hippocampus; light labeling was present in an adjacent structure, the reticular thalamic nucleus (Fig. 6*E*).

DISCUSSION

In the present study, we have used electrophysiology and autoradiography and have compared two related species. We found that (*i*) in the rat, hippocampal nonpyramidal neurones, located in field CA1 and in the CA1/subiculum transition region, are excited by oxytocin, whereas in the guinea pig, similar hippocampal nonpyramidal neurones are unaffected by oxytocin—although they are excited by carbachol; and (*ii*) oxytocin binding sites are present in the rat hippocampus in the CA1 and in the subiculum, whereas no oxytocin binding sites could be detected in the guinea pig hippocampus. We conclude that central oxytocin binding sites, or at least part of them, represent functional neuronal receptors. Thus, autoradiography appears to be a valid means of mapping areas in the brain where oxytocin may be presumed to act as a neurotransmitter (6, 7). Oxytocin immunocytochemistry appears less predictive in this respect (8).

A correlation between the density of binding sites and the magnitude of the biological effect has been shown to exist for few other neurotransmitters (13–15). These studies, however, were not based on species-specific differences but

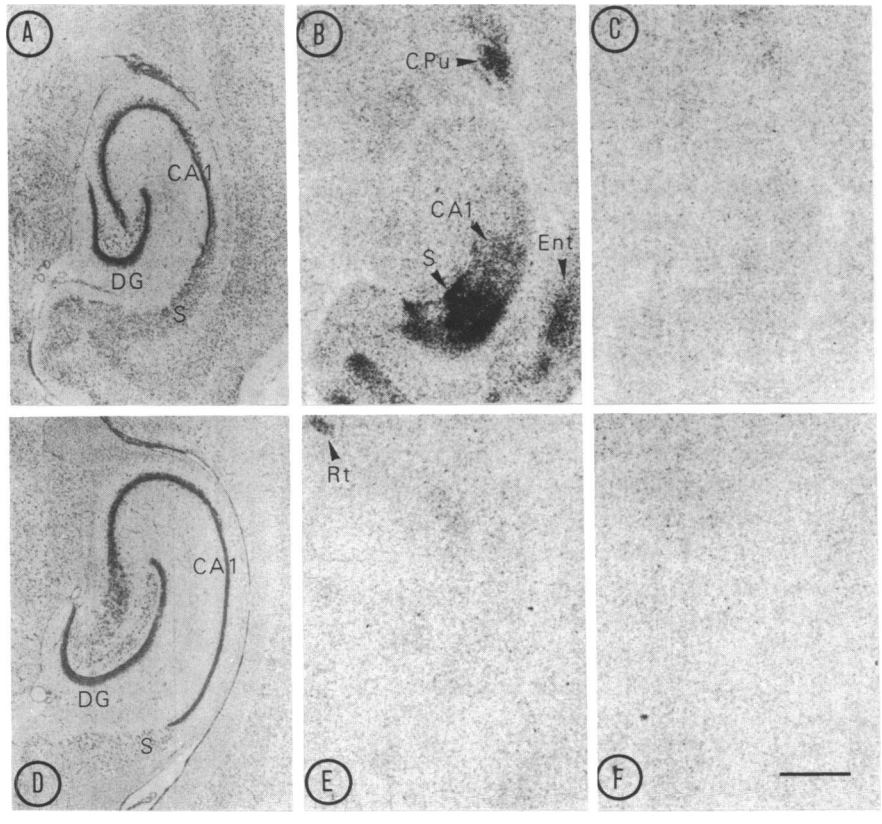

FIG. 6. Localization of oxytocin binding sites in the rat (*A–C*) and guinea pig (*D–F*) hippocampus. For each species, adjacent horizontal brain sections are shown. (*A* and *D*) Cresyl violet-stained sections showing the hippocampal region and some of its major subdivisions: subiculum (S), field CA1 pyramidal cells (CA1), dentate gyrus (DG). (*B* and *E*) Autoradiographs obtained following incubation of sections with 0.05 nM radioiodinated oxytocic antagonist. (*C* and *F*) Same as *B* and *E*, except that 100 nM unlabeled oxytocin was added to the incubation medium. Ent, entorhinal cortex; CPu, caudate putamen; Rt, reticular thalamic nucleus. (Bar = 1 mm.)

rather compared different brain regions in one and the same species.

The synthetic structural analogue des-Gly(NH_2)-d(CH_2)$_5$-[Tyr(Me)^2,Thr^4]OVT acts *in situ* as a potent, selective oxytocin antagonist. The *in vitro* pA_2 (negative logarithm of antagonist concentration producing a 2-fold shift of the agonist concentration–response curve) for its antioxytocic activity in the presence of 0.5 mM Mg^{2+} is 8.24, whereas that for its antivasopressor activity is 6.48 and that for its antiantidiuretic activity is 5.3 (9). We have found that the excitatory action of oxytocin on rat hippocampal nonpyramidal neurones was suppressed by this structural analogue. This result confirms that the neuronal action of oxytocin in the rat hippocampus is mediated by oxytocin receptors similar to those found on peripheral target tissues and thus confirms previous studies (4, 16).

Specific oxytocin binding sites in the rat hippocampus were visualized by making use of a synthetic structural analogue, d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr(NH_2)^9]OVT, radioiodinated on the tyrosylamide residue at position 9 (10). Except for this last residue, this ligand is similar in sequence to the antagonist used in electrophysiological experiments; like the latter, it has a potent *in situ* antioxytocin activity and it has been shown to bind to uterine oxytocin receptors *in vitro* with high affinity and high selectivity (10). Thus, both compounds probably interact with the same population of hippocampal oxytocin receptors.

Autoradiography showed that in the hippocampal region of the rat labeling was most intense in the subiculum, whereas in the field CA1 and in the CA1/subiculum transition region—from which the electrophysiological recordings were obtained—moderate to light labeling was present. This calls for the following remarks. (*i*) The high density of oxytocin binding sites in the rat subiculum may lead one to predict that neurones responsive to oxytocin are present in this brain area. This remains to be proven, however. Preliminary experiments indicated that subicular neurones are apparently devoid of spontaneous firing activity—a fact that renders extracellular recordings unrewarding—and stable intracellular impalements from subicular neurones were seldom obtained. (*ii*) The relative weakness of labeling observed in the rat CA1 and CA1/subiculum transition region apparently contrasts with the potency of the neuronal excitation brought about by oxytocin in these areas. However, the present as well as previous electrophysiological studies (3, 4) indicate that, in the rat, only nonpyramidal neurones respond directly to oxytocin. Hippocampal interneurones—to which nonpyramidal oxytocin-sensitive CA1 cells belong—have been estimated to represent only 2–4% of the whole hippocampal neuronal population (17). Thus, if oxytocin receptors were exclusively, or preferentially, located on oxytocin-responsive neurones, a relatively low density of oxytocin binding sites in field CA1 is expected.

The hippocampal region (i.e., the hippocampus proper, the dentate gyrus, and the subiculum) receives, via the fimbria-fornix, a dense cholinergic innervation arising from the medial septal nucleus and the nucleus of the diagonal band (18). Cholinergic nerve terminals make synaptic contacts with hippocampal principal cells—pyramidal and granule cells—as well as with hippocampal interneurones (19, 20). Electrophysiological studies have shown that acetylcholine acts as an endogenous neurotransmitter on pyramidal neurones (21, 22) and that it causes excitation by modulating various membrane currents (23). Exogenous acetylcholine, however, also has a presynaptic inhibitory effect, both in field CA1 (24–26) and in the dentate gyrus (27), which may be explained, at least in part, by an excitatory action on inhibitory interneurones. Thus, morphological and electrophysiological evidence points to the existence of cholinoceptive hippocampal interneurones. Our results support this notion, by showing that in the rat oxytocin-responsive nonpyramidal CA1 cells are directly excited by cholinomimetic compounds and that this excitation is mediated by receptors of the muscarinic type.

This and previous studies indicate that in the rat hippocampal nonpyramidal neurones are the target of a variety of excitatory and inhibitory neurotransmitters: they respond to oxytocin, to muscarinic cholinergic agonists, to tachykinins and bombesin (28), and to μ-type opioids (29). Surprisingly, the homologous hippocampal interneurones from the guinea pig differ in their chemosensitivity, as they do not respond to oxytocin. This and a similar difference in oxytocin sensitivity of rat and guinea pig vagal neurones (8) indicate that this peptide may be a neurotransmitter in some species but not in others or, more likely, that it may act in different brain regions in different species.

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