Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3926–3930, June 1987
Neurobiology

Neurons in the dorsal motor nucleus of the vagus nerve are excited by oxytocin in the rat but not in the guinea pig

(brain slices/electrophysiology/immunohistochemistry/Lucifer yellow injection/neuropeptides)

M. RAGGENBASS, M. DUBOIS-DAUPHIN, S. CHARPAK, AND J. J. DREIFUSS*

Department of Physiology, University Medical Center, 1211 Geneva 4, Switzerland

Communicated by Ewald R. Weibel February 24, 1987 (received for review October 9, 1986)

ABSTRACT **Intracellular recordings were obtained from vagal neurons and their response to oxytocin was investigated in slices from the rat and the guinea pig brainstem. After recording, Lucifer yellow was injected into the cells to verify their localization within the dorsal motor nucleus of the vagus nerve (dmnX). In the rat, virtually all neurons throughout the rostrocaudal extent of the dmnX increased their rate of firing in the presence of 10–1000 nM oxytocin and their membrane depolarized in a reversible concentration-dependent manner. This excitation was probably exerted directly on the impaled cells rather than being synaptically mediated, since it persisted in a low calcium/high magnesium medium or in the presence of tetrodotoxin. These data provide evidence for a direct membrane effect of oxytocin on a defined population of neurons in the rat brain. In the guinea pig, vagal neurons were fired by glutamate but were not excited by oxytocin, even though we detected many more oxytocin-immunoreactive structures in the guinea pig dmnX than in the rat dmnX. Therefore, homologous nuclei in the brains of two closely related mammals differ markedly in the density of oxytocinergic axons they contain. Unexpectedly, the magnitude of the electrophysiological effects of oxytocin on vagal neurons appeared inversely related to the amount of oxytocin-like immunoreactivity present in dmnX.**

In recent years, morphological and physiological evidence has accumulated indicating that vasopressin and oxytocin, in addition to the functions they exert as circulating hormones in mammals, may also act as neurotransmitters and/or neuromodulators in various areas of the central nervous system (1, 2). In both the nucleus of the solitary tract (nTS) and in the dorsal motor nucleus of the vagus nerve (dmnX) of rats vasopressin- and oxytocin-containing axons have been detected (1, 3). These axons originate, at least in part, from neurons located in the paraventricular nucleus of the hypothalamus (4, 5). Vasopressin and oxytocin can be released *in vitro* from rat brainstem tissue in a calcium-dependent manner (6). Moreover, using coronal slices from rat brainstem, Charpak *et al.* (7) provided electrophysiological evidence that neurons located in the region of the dmnX respond to oxytocin as well as vasopressin, and they suggested that these neurons are endowed with oxytocin receptors. Although some of the effects they observed may have been indirect, their data support the notion that endogenous oxytocin may function as a neurotransmitter/neuromodulator in the dorsal brainstem of rats.

In the present study, a combined electrophysiological, pharmacological, and histological approach was used on rat brainstem slices to assess the location and the morphology of a class of neurons that are directly responsive to oxytocin. A parallel study was carried out in the guinea pig. In addition, immunohistochemical methods were used to compare the amount of oxytocin-immunoreactive material present in the dmnX–nTS complex of these two species.

MATERIALS AND METHODS

Brainstem Slices. Experiments were performed using male adult rats from a Sprague–Dawley-derived strain (200–300 g) and male adult guinea pigs (300–500 g). Rats and guinea pigs were decapitated, the skull was opened, and the brain was gently excised. The cerebellum, the midbrain, and the forebrain were removed and a block of brainstem tissue was prepared by making two transverse cuts—caudally to the inferior colliculus and rostrally to the first pair of cervical nerves. An additional longitudinal cut was made in a parasagittal plane and the exposed surface was fixed with cyanoacrylate to the stage of a vibrating microtome (Campden Instruments, London, U.K.). Two or 3 parasagittal slices (300–400 μm thick), which included the dmnX, the nTS, and the nucleus of the hypoglossal nerve, were obtained from each animal. The slices were transferred to a thermoregulated (34°C–35°C) recording chamber and placed on a polyester grid at the interface between a humidified oxygenated atmosphere and a perifusion solution containing 130 mM NaCl, 5 mM KCl, 20 mM $NaHCO_3$, 2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 10 mM glucose, and 2 mM $CaCl_2$, gassed with 95% O_2/5% CO_2 (pH 7.35–7.45). The perifusion solution flowed at 2 ml/min and could be completely exchanged in ≈2.5 min. Before the recordings were started, the preparation was allowed to recover for 1–3 hr at 34°C–35°C. To achieve synaptic uncoupling, we added 0.5–2.5 μM tetrodotoxin or used a modified medium that contained 0.2 mM $CaCl_2$ and 6 mM $MgSO_4$ instead of the usual concentrations of calcium and magnesium.

Electrophysiological Recordings. Intracellular recordings were obtained using glass micropipettes filled with either 3 M potassium acetate (pH 7.4) or 3 M KCl and having tip d.c. resistances ranging from 40 to 120 MΩ. Voltage signals were amplified, displayed on an oscilloscope, and stored on magnetic tape. Membrane potential and rate meter records of cell firing were monitored on a chart recorder. Synaptic activity of neurons in the dmnX was elicited by stimulation at the border between the dmnX and the nTS. Bipolar stimulation electrodes were made of twisted nichrome wires (diameter, 100 μm) and were isolated except for their tips. The stimuli consisted of constant current pulses (50–300 μA, 0.1 ms) delivered at frequencies of 0.2 Hz or less.

Lucifer Yellow Staining. In several experiments, intracellular recordings were carried out using micropipettes filled with a solution of the fluorescent dye Lucifer yellow (5% in 0.3 M LiCl; tip resistance, 200–350 MΩ). At the end of the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: dmnX, dorsal motor nucleus of the vagus nerve; nTS, nucleus of the solitary tract.

*To whom reprint requests should be addressed at: Department of Physiology, University Medical Center, 9 Avenue de Champel, CH 1211 Geneva 4, Switzerland.

recording session, the neurons were injected by passing negative current pulses through the micropipette (0.2–0.5 nA, 500 ms) at a frequency of 1 Hz for 1–5 min. The slices were fixed for at least 12 hr in a 4% paraformaldehyde solution in phosphate-buffered saline (PBS; pH 7.3) containing 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4. They were then immersed in a 30% (wt/vol) sucrose solution in PBS, to ensure cryoprotection of the tissue. In a cryostat, 60-μm-thick sagittal sections were cut, mounted on gelatin-coated slides, air-dried, and counterstained with ethidium bromide at 6 mg/liter in PBS. They were examined with an epifluorescence microscope (Orthoplan, Leitz, F.R.G.), equipped with H2 or I2 filters.

Immunohistochemistry. Animals were anesthetized with sodium pentobarbital (500 mg/kg, i.p.) and perfused through the left ventricle with 250 ml of isotonic saline followed by 800 ml of 4% paraformaldehyde in PBS at room temperature. The brain and the cervical spinal cord were removed and kept in the fixative solution at 4°C for 2 days. After transfer into a 30% sucrose solution in PBS, 60-μm-thick parasagittal sections were cut in a cryostat. The sections were then rinsed in PBS for 30 min and incubated for 36 hr with oxytocin antiserum at a dilution of 1:1000 in PBS without hydrogen peroxide pretreatment. At the end of the reaction, they were rinsed in PBS, incubated for 60 min in goat anti-rabbit antiserum (Nordic, Tilbury, Netherlands) at a dilution of 1:50 in PBS, rinsed again, and incubated for 90 min with peroxidase-antiperoxidase (UCB-Bioproducts, Braine L'Alleud, Belgium) at a dilution of 1:100 in PBS. All solutions contained 0.3% Triton X-100 and 0.1 M L-lysine. Incubations were carried out at 4°C under constant shaking. The peroxidase activity was revealed using diaminobenzidine as substrate. The sections were incubated for 10 min with 0.05% diaminobenzidine in 0.1 M phosphate buffer (pH 7.3); 0.012% peroxide was then added and the incubation continued for 20 min. The sections were rinsed in a 2% sodium acetate solution, treated to intensify the diaminobenzidine reaction product (8), rinsed again in PBS, and mounted on chromalun-gelatin coated slides.

In control experiments, brain sections were prepared and all immunohistochemical steps were carried out as described above, except that the oxytocin antiserum was preabsorbed with oxytocin at 10 μg/ml. Following this procedure, no immunoreactive nerve fibers and terminals could be detected in the dmnX–nTS complex.

Chemicals. Oxytocin and [Arg^8]vasopressin were purchased from Bachem Fine Chemicals. Tetrodotoxin was from Calbiochem and sodium L-glutamate was from Merck. These substances were tested by dissolving them in the perifusion solution. Lucifer yellow was obtained from Fluka (Buchs, Switzerland). Oxytocin antiserum was a gift from G. Nilaver (Department of Neurology, Columbia University, New York).

RESULTS

Effect of Oxytocin on Rat Vagal Neurons. Stable intracellular recordings were obtained from neurons in the dmnX region. Neurons had resting membrane potentials in excess of −50 mV (up to −80 mV), an average input resistance of 145 MΩ (range, 77–350 MΩ; $n = 25$) and fired spontaneously at a frequency of 1.1 ± 0.2 spikes per s ($n = 41$; mean ± SEM). Oxytocin excited 39 of 41 neurons in a reversible concentration-dependent manner, the remaining cells being unaffected. At 1 μM, the peptide caused a mean increase in firing, above the resting level, of 3.5 ± 0.5 spikes per s ($n = 32$) and a membrane depolarization of 12.5 ± 2.2 mV ($n = 5$). At 0.1 μM, the firing rate increased by 2.9 ± 0.7 spikes per s ($n = 7$) and the neurons depolarized by 8.5 ± 1.9 mV ($n = 5$). At 0.01 μM, the increase in firing was 1.7 ± 0.9 spikes per s ($n = 3$).

To assess the location and the morphology of the oxytocin-responsive neurons, intracellular recordings were carried out with Lucifer yellow-filled micropipettes. Of 21 neurons in 21 slices, 20 were excited by oxytocin at 0.1–1 μM (Fig. 1). At the end of the recording session, the neurons were injected with the fluorescent dye and the slices were fixed and processed for histological analysis. Twenty-one stained neurons could be visualized. All of them, including the unresponsive one, were located within the boundaries of the dmnX (Fig. 2 *A* and *B*); they were bipolar or multipolar neurons and had a triangular or spindle-shaped cell body. Their long axis ranged from 24 to 48 μm and their short axis ranged from 15 to 34 μm. They were apparently randomly distributed within the nucleus.

The action of oxytocin was assumed to be postsynaptic, as this peptide depolarized neurons in the dmnX area even in conditions in which synaptic transmission was suppressed. Synaptic uncoupling was achieved either by replacing the normal medium with a low calcium/high magnesium medium or by perfusing the preparation with a solution containing 0.5–2.5 μM tetrodotoxin. Twelve of 12 neurons (3 with Lucifer yellow-filled micropipettes) retained their sensitivity to oxytocin in the low calcium/high magnesium medium (Fig. 3) and 21 of 21 neurons (8 with Lucifer yellow-filled micropipettes) still responded to oxytocin in the presence of tetrodotoxin.

Insensitivity of Guinea Pig Vagal Neurons to Oxytocin. Similar to rat vagal neurons, guinea pig neurons in the dmnX fired spontaneously at low frequency (1.8 ± 0.2 spikes per s; $n = 24$), had resting membrane potentials greater than −50 mV (up to −75 mV), and had an average input resistance of 120 MΩ (range, 28–260 MΩ; $n = 14$). In contrast to the results obtained from rat slices, none of the 21 neurons tested was affected by oxytocin at 1 μM. However, their firing increased when 5–8 mM glutamate was added to the perifusion solution (Fig. 4). Ten such oxytocin-insensitive neurons were recorded in 10 slices and injected with Lucifer yellow. The 9 successfully stained neurons were all located in the dmnX. The long axis of their cell bodies ranged from 29 to 48 μm, the short axis ranged from 19 to 38 μm (Fig. 2 *C* and *D*).

Oxytocin Immunoreactivity in the dmnX–nTS Area of the Rat and the Guinea Pig. In the rat brainstem, oxytocin-like immunoreactivity was conspicuous in nTS (Fig. 5*A*). In the

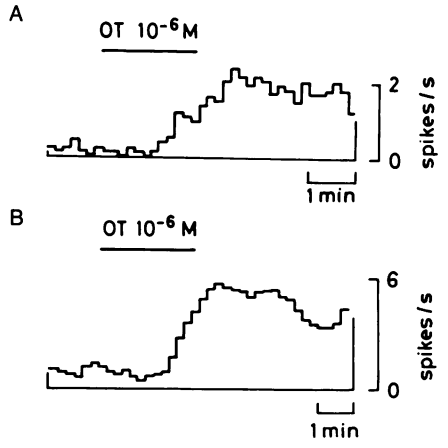

FIG. 1. Effects of oxytocin (OT) on two rat vagal neurons. OT (1 μM) was applied for the duration indicated by the horizontal bar above each rate meter record. Increases in firing rate were 2 (*A*) and 4.5 (*B*) spikes per s above the resting level. Lucifer yellow-filled micropipettes were used. Both neurons, injected at the end of the recording with Lucifer yellow, were located within the dmnX (see Fig. 2 *A* and *B*).

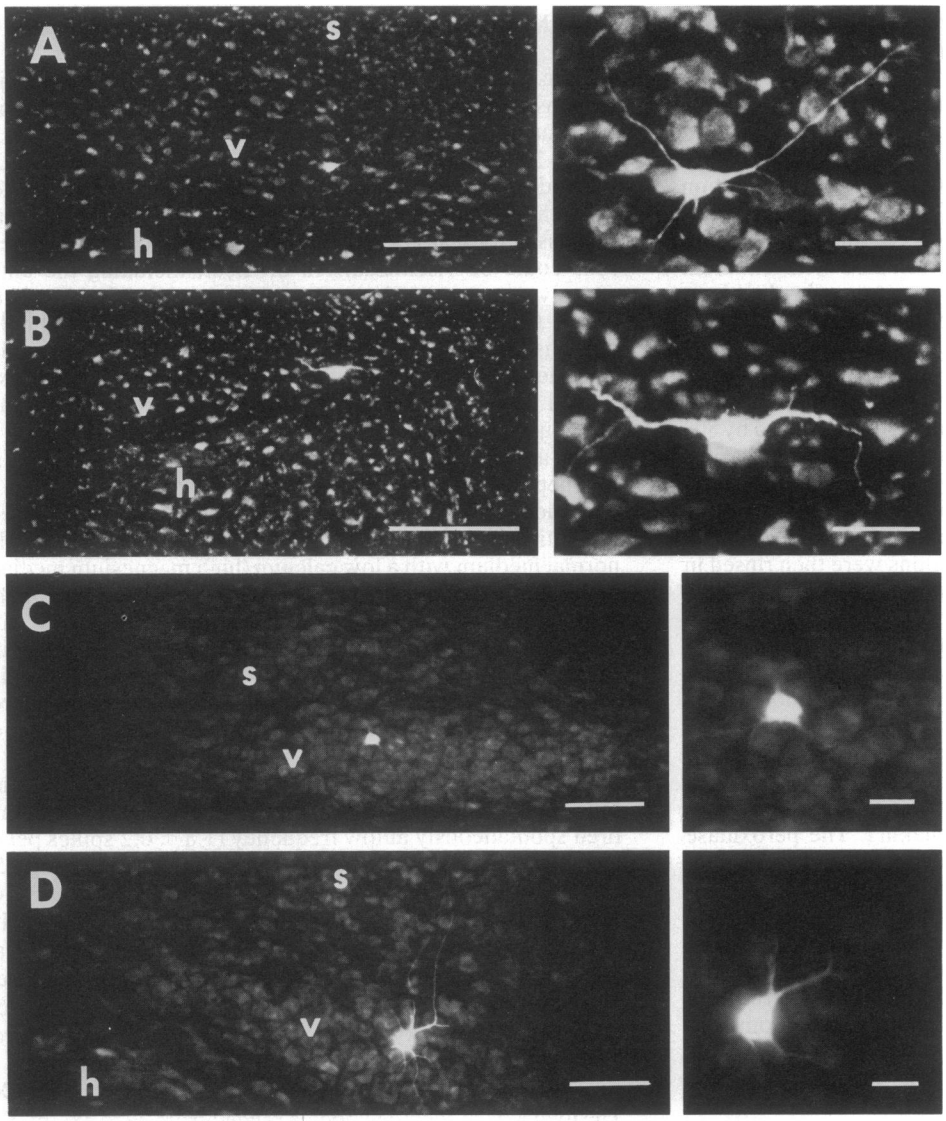

FIG. 2. Lucifer yellow-labeled vagal neurons in parasagittal sections of the rat and guinea pig brainstem. Intracellular recordings were obtained from four cells (*A–D*) during oxytocin application and prior to Lucifer yellow injection. Neurons in *A* and *B* are from rat slices; their response to oxytocin is shown in Fig. 1. Neurons in *C* and *D* are from guinea pig slices; their response to glutamate and lack of response to oxytocin are illustrated in Fig. 4. In each section, the same area viewed by fluorescence microscopy is shown at low (*Left*) and high (*Right*) magnification. (Bars: *Left*, 200 μm; *Right*, 50 μm.) After counterstaining with ethidium bromide, air-drying, and clearing in toluol, slices in *A* and *B* were embedded in Entellan (Merck), whereas slices in *C* and *D* were immersed in a glycerol/PBS solution (Citifluor AF1) without prior dehydration and clearing. The latter procedure, by minimizing cell shrinkage, allowed for better tissue preservation and enhanced the contrast between the injected neuron and the surrounding unlabeled cells. Note that all four labeled neurons are located within the dorsal motor nucleus of the vagus nerve (v); s, nucleus of the solitary tract; h, nucleus of the hypoglossal nerve.

adjacent dmnX, however, we observed only few oxytocin-immunoreactive structures; an occasional beaded axon was seen (Fig. 5*C*). In the guinea pig dmnX, on the other hand, the oxytocin-like immunoreactive structures were far more numerous than in the rat dmnX. At low magnification, the dmnX, filled with reaction product, could be clearly distinguished from the adjacent unreactive cell groups (Fig. 5*B*). At high magnification, immunoreactivity appeared as axon terminal-like dots surrounding the dmnX cell bodies (Fig. 5*D*).

DISCUSSION

We have examined the effect of oxytocin on Lucifer yellow-injected dmnX neurons in slices from the rat and the guinea pig brainstem. In the rat, virtually all dmnX neurons were excited by oxytocin. Surprisingly, vagal neurons from a closely related species, the guinea pig, were not fired by oxytocin.

Oxytocin Effects in the Rat dmnX. The present study showed, first, that a defined population of rat central neurons are depolarized by oxytocin; this action was direct—i.e., unrelated to calcium-dependent synaptic transmission—since it persisted in conditions of synaptic uncoupling. In a previous study, Charpak *et al.* (7) found that the effect of oxytocin in the dmnX region of rats could be reversibly suppressed by a structural analogue known to act as an oxytocin and a vasopressin antagonist and could be mimicked by a selective oxytocin agonist. These data and the results of the present work suggest that rat vagal neurons possess oxytocin receptors. Second, we found that in the rat, all the Lucifer yellow-injected neurons were of medium to large size and were distributed over the whole rostrocaudal extent of the dmnX. This suggests that the oxytocin-sensitive vagal neurons were mostly preganglionic motoneurons, projecting toward cervical, thoracic, and abdominal visceral areas. Indeed, although in the cat two distinct types of dmnX neurons have been identified based on cell size and axon projections (9, 10), no small nonpreganglionic vagal neurons have been found in the dmnX of rats (11–14) and virtually all neurons in this nucleus are retrogradely labeled following horseradish peroxidase injection into the vagus nerve (15, 16). Third, we showed that oxytocin-immunoreactive fibers, albeit scarce, are present in the rat dmnX, thus confirming previous results by other workers (see Introduction). Accordingly, the neuronal excitation brought about by oxytocin perifusion may mimic the action of endogenous oxytocin on rat vagal neurons.

Although further experiments are needed to substantiate the notion that oxytocin may function as a neurotransmitter/neuromodulator in the dmnX of rats, one may speculate that modulation of the firing of vagal motoneurons by oxytocinergic pathways could influence cardiac, respiratory, and gastrointestinal activity in this species.

Previous studies showed that oxytocin can affect neuronal firing in various regions of the rat central nervous system (2). Morris *et al.* (17) found that oxytocin applied microionto-

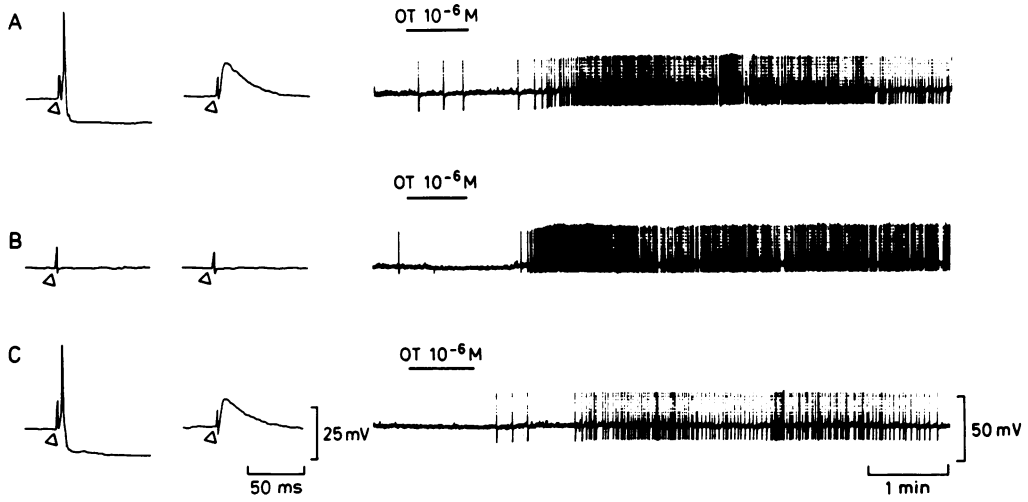

FIG. 3. Effects of oxytocin (OT) on a rat vagal neuron in normal medium and under conditions of synaptic uncoupling. Membrane potential recordings in normal medium (*A*), in low calcium/high magnesium medium (*B*), and again in normal medium (*C*). (*Right*) OT (1 μM) was applied during the 1-min period indicated by the horizontal bar above each trace. Action potentials are reduced in amplitude because of the low-frequency response of the chart recorder used. (*Left*) Response to electrical stimuli (artefacts are marked by triangles) was recorded 1–2 min before each application of OT. In *A* and *C*, synaptic coupling was evidenced by monitoring orthodromically evoked action potentials and excitatory postsynaptic potentials, unmasked by hyperpolarizing the neuronal membrane by 20 mV. In *B*, synaptic uncoupling was achieved, as shown by disappearance of the evoked potentials; however, the neuron was strongly excited by OT. Resting membrane potential, −50 mV; input resistance, 170 MΩ. Potassium acetate-filled micropipettes were used.

phoretically could excite or inhibit neurons in the rat caudal medulla. Their results obtained after application of oxytocin antagonists as well as the results of the present study suggest, however, that the inhibition observed by Morris *et al.* (17) may have been due to indirect or unspecific effects of oxytocin. Mühlethaler *et al.* (18, 19) showed that oxytocin and vasopressin exerted an excitatory action in the rat hippocampus, an effect mediated by oxytocin receptors. Using indirect electrophysiological criteria, they concluded that the target cells were probably inhibitory interneurons, but intracellular recordings would be needed to confirm this conjecture.

Oxytocin and the Guinea Pig dmnX. On the basis of their location and morphology, there is little doubt that most, if not all, of the neurons we recorded in the guinea pig were preganglionic vagal motoneurons. In addition, they exhibited electrophysiological properties similar to those described by Yarom *et al.* (20), who identified vagal motoneurons by antidromic invasion in guinea pig brainstem slices.

Surprisingly, in contrast to what was found in the rat,

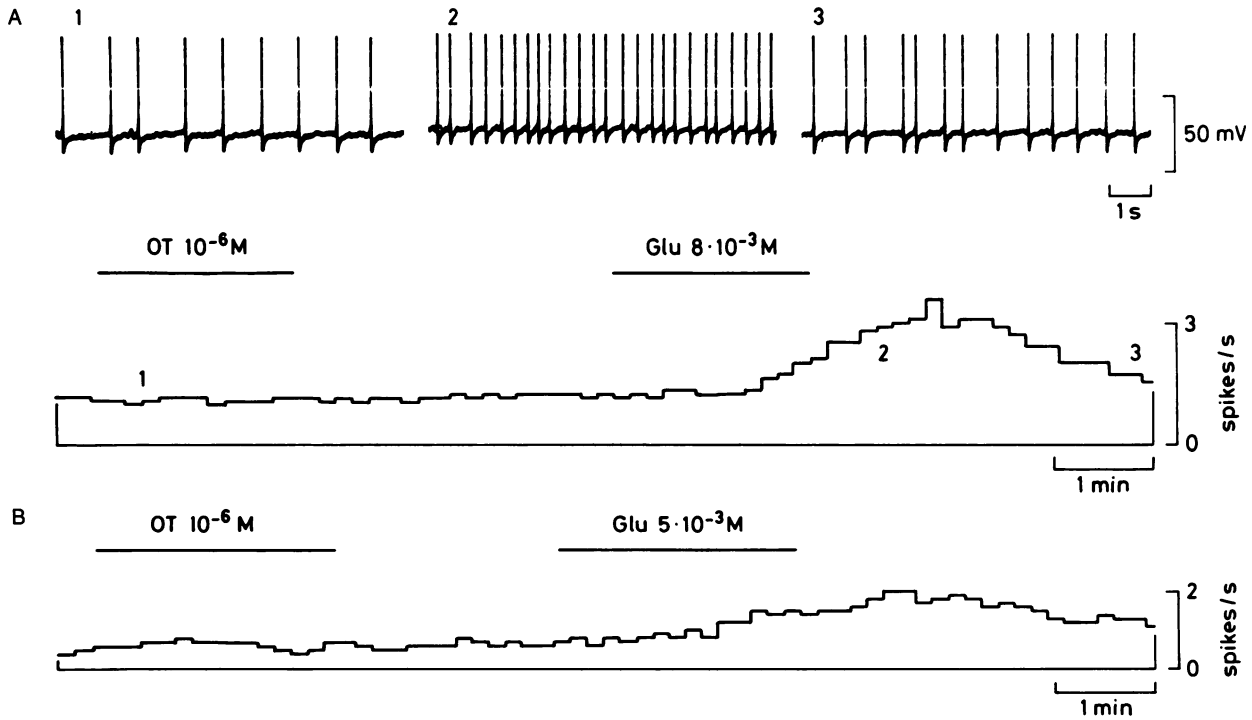

FIG. 4. Effects of oxytocin (OT) and of glutamate (Glu) on two guinea pig vagal neurons. (*Upper*) Photographs of action potentials of cell A; (*Middle* and *Lower*) rate meter records of cells A and B. Duration of application (horizontal bar) and concentration of drugs are indicated above each rate meter record. In *A*, action potentials were recorded at the times (*1–3*) indicated on the rate meter record. Lucifer yellow-filled micropipettes were used. Both neurons were injected with Lucifer yellow and were found to be in the dmnX; their morphology and location are shown in Fig. 2 *C* and *D*.

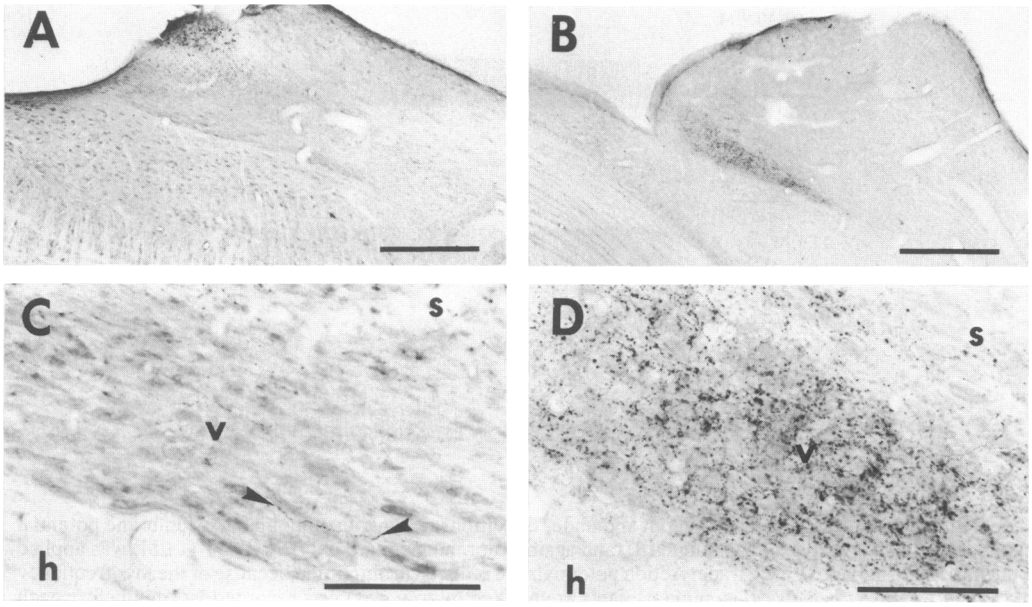

FIG. 5. Oxytocin immunohistochemical staining of parasagittal sections of the rat (*A*) and guinea pig brainstem (*B*). (*C* and *D*) Higher magnifications of parts of *A* and *B*, respectively, centered on the dorsal motor nucleus of the vagus nerve (v). s, Nucleus of the solitary tract; h, nucleus of the hypoglossal nerve. In the rat, only a few long and beaded axons were immunoreactive (arrowheads in *C*), whereas in the guinea pig reaction product was much more abundant (*D*). Bars: *A* and *B*, 0.5 mm; *C* and *D*, 0.1 mm.

guinea pig dmnX neurons were not excited by oxytocin. However, the dmnX in the guinea pig contained a high amount of immunoreactive oxytocin. Mismatch between the level of neurotransmitters (as detected by immunohistochemistry or by radioimmunoassay) and the density of their receptors (as determined by the specific binding of radioligands) has been described in various regions of the mammalian central nervous system (see, for example, refs. 21 and 22). In the present study, a marked discrepancy was found when comparing the presence or absence of an electrophysiological response of vagal neurons to oxytocin with the amount of oxytocin-like immunoreactivity present in rat and guinea pig dmnX. Assuming that the antiserum used in this study recognizes oxytocin selectively, several possibilities might be considered to explain the mismatch we observed. (*i*) The quantity of oxytocin present in axons at a given site in the nervous system may depend primarily on the balance between production and release, rather than reflect the efficiency with which the neuropeptide acts locally as a neurotransmitter. (*ii*) In the dmnX of the guinea pig, oxytocin may have either glial cells or endothelial cells as target or, if affecting neurons, might cause long-term modifications in cell function that are not accompanied by rapid and early changes in membrane properties. (*iii*) It is also conceivable that oxytocin receptors may be located presynaptically with respect to the vagal motoneurons, since in the rat superior cervical ganglion, arginine vasopressin has been recently shown to reduce the amplitude of the fast excitatory postsynaptic potential by a presynaptic action (23).

Unless due to some systematic bias of unknown origin we missed an oxytocin-sensitive subpopulation of vagal guinea pig neurons, the main conclusion of our study is that while in the rat virtually all vagal motoneurons are depolarized by oxytocin, those in the guinea pig are not. Thus, homologous populations of central neurons from two closely related species differ widely in their response to a neuropeptide. This suggests that caution should be exerted in extrapolating data from one species to another.

We thank Ms. A. Marguerat and D. Machard for technical assistance and Ms. A. Cergneux for secretarial work. This work was supported in part by the Swiss National Science Foundation (Grants 3.560.1.83 and 3.358.0.86) and the Fondation Carlos and Elsie de Reuter.

1. Sofroniew, M. V. (1985) in *Handbook of Chemical Neuroanatomy: GABA and Neuropeptides in the CNS*, eds. Björklund, A. & Hökfelt, T. (Elsevier, Amsterdam), Vol. 4, pp. 93–165.
2. Mühlethaler, M., Raggenbass, M. & Dreifuss, J. J. (1985) in *Neurotransmitter Actions in the Vertebrate Nervous System*, eds. Rogawski, M. A. & Barker, J. L. (Plenum, New York), pp. 439–458.
3. Kalia, M., Fuxe, K., Hökfelt, T., Harfstrand, A., Lang, R. E. & Ganten, D. (1984) *Brain Res.* **321,** 71–82.
4. Sofroniew, M. V. & Schrell, U. (1981) *Neurosci. Lett.* **22,** 211–217.
5. Sawchenko, P. E. & Swanson, L. W. (1982) *J. Comp. Neurol.* **205,** 260–272.
6. Buijs, R. M. & Van Heerikhuize, J. J. (1982) *Brain Res.* **252,** 71–76.
7. Charpak, S., Armstrong, W. E., Mühlethaler, M. & Dreifuss, J. J. (1984) *Brain Res.* **300,** 83–89.
8. Liposits, Z., Sétáló, G. & Flerkó, B. (1984) *Neuroscience* **13,** 513–525.
9. McLean, J. H. & Hopkins, D. A. (1981) *J. Comp. Neurol.* **195,** 157–175.
10. McLean, J. H. & Hopkins, D. A. (1982) *J. Comp. Neurol.* **206,** 243–252.
11. Kalia, M., Fuxe, K., Hökfelt, T., Johansson, O., Lang, R., Ganten, D., Cuello, C. & Terenius, L. (1984) *J. Comp. Neurol.* **222,** 409–444.
12. Kalia, M. & Fuxe, K. (1985) *J. Comp. Neurol.* **233,** 285–307.
13. Kalia, M., Fuxe, K. & Goldstein, M. (1985) *J. Comp. Neurol.* **233,** 308–332.
14. Kalia, M., Fuxe, K. & Goldstein, M. (1985) *J. Comp. Neurol.* **233,** 333–349.
15. Leslie, R. A., Gwyn, D. G. & Hopkins, D. A. (1982) *Brain Res. Bull.* **8,** 37–43.
16. Kalia, M. & Sullivan, J. M. (1982) *J. Comp. Neurol.* **211,** 248–264.
17. Morris, R., Farmery, S. M., Roberts, C. J. & Hill, R. G. (1984) *Neurosci. Lett.* **48,** 161–166.
18. Mühlethaler, M., Sawyer, W. H., Manning, M. M. & Dreifuss, J. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6713–6717.
19. Mühlethaler, M., Charpak, S. & Dreifuss, J. J. (1984) *Brain Res.* **308,** 97–107.
20. Yarom, Y., Sugimori, M. & Llinás, R. (1985) *Neuroscience* **16,** 719–737.
21. Mantyh, P. W. & Hunt, S. P. (1986) *J. Neurosci.* **6,** 1537–1544.
22. McLean, S., Rothman, R. B. & Herkenham, M. (1986) *Brain Res.* **378,** 49–60.
23. Kiraly, M., Audigier, S., Tribollet, E., Barberis, C., Dolivo, M. & Dreifuss, J. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 5335–5339.