Neuroscience Letters, 33 (1982) 79–84
Elsevier Scientific Publishers Ireland Ltd.

THE EFFECT OF MICROIONTOPHORETICALLY APPLIED VASOPRESSIN AND OXYTOCIN ON SINGLE NEURONES IN THE SEPTUM AND DORSAL HIPPOCAMPUS OF THE RAT

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(Received July 7th, 1982; Revised version received and accepted August 30th, 1982)

Key words: lateral septum – hippocampus – vasopressin – oxytocin – microiontophoresis – rat

When arginine[8]-vasopressin (AVP) or oxytocin (OXT) were applied microiontophoretically to neurones in the lateral septal complex and dorsal hippocampus of rats, more than 50% of the neurones responded with excitation. The remaining neurones were not affected by AVP or OXT. Both the AVP- and OXT-induced responses displayed a short latency in onset and offset. Medial septal neurones were almost never excited by AVP or OXT. In more than 50% of the lateral septal and hippocampal neurones, the responses to glutamate were enhanced during the iontophoretic release of AVP or OXT. The possible role of both peptides as neurotransmitters in the structures examined is discussed.

Recent immunocytochemical, electron microscopic and biochemical studies suggest that the two closely related neuropeptides of hypothalamic neurosecretory origin in rats, arginine[8]-vasopressin (AVP) and oxytocin (OXT), might function as neurotransmitter in various regions of the brain, including the lateral septal complex (LSC) and hippocampus. Pathways containing immunoreactive AVP and OXT were traced from the suprachiasmatic and paraventricular nuclei towards the septum [3, 15, 16] and hippocampus [1]. Some of the synaptic-like terminals which were observed on or in the vicinity of the LSC neurones contain granules immunoreactive with antibodies raised against AVP [2]. Moreover, AVP and OXT were detected by radioimmunoassay in the septum and hippocampus [5–7].

Microinjection of AVP and OXT into the septum resulted in a similar effect of both peptides on the delay in extinction of avoidance behaviour [11] and on the acceleration of the hippocampal theta rhythm generated by rats during paradoxical sleep [18]. These findings suggest that AVP and OXT may exert a similar action on septal neurones.

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In the present study we examined whether iontophoretically applied AVP and OXT could alter the spontaneous activity of hippocampal and septal neurones and whether the effects of these peptides were qualitatively similar. Recent biochemical, pharmacological and electrophysiological evidence [9, 19] suggest that glutamate (Glu) or a closely related excitatory amino acid functions as neurotransmitter in fimbria-fornix fibres innervating the LSC neurones. We therefore also examined the effect of AVP and OXT on the Glu-induced excitation in LSC neurones.

The experiments were performed on 28 male Wistar rats (250–300 g), anaesthetized with urethane (1.5 g/kg, intraperitoneally). Following extensive craniotomy, the cortex and corpus callosum overlying the septal area and the dorsal hippocampus were removed by suction. Action potentials of single neurones were recorded extracellularly through a single glass micropipette (containing 3 M NaCl) glued to a 7-barrelled drug pipette (tip size ca 10 μm) so that the recording tip protruded in front of the multibarrelled pipette by 30–50 μm. The following drugs were applied by microiontophoresis: L-glutamic acid (Sigma, 0.25 M, pH 8), arginine^8-vasopressin (Organon, batch number TH 891, 408 U/mg, 5 mM in 165 mM NaCl, pH 5–6), oxytocin (Organon, Org 4882, 567 U/mg, 5 mM in 165 mM NaCl, pH 5–6). One barrel contained 165 mM NaCl. The current was balanced automatically through a 3 M NaCl-filled barrel of the multibarrelled pipette during all microiontophoretic tests. The retaining current for Glu and 165 mM NaCl was 10 nA: very low (0–5 nA) retaining currents were used for the peptides. Conventional techniques were used for extracellular recordings and iontophoretic applications. The neurones encountered in the septum were classified as lateral or medial septal (MS) cells according to their response to electrical stimulation of fimbria-fornix fibres. LSC neurones exhibited a short-latency orthodromic response to these stimuli. MS neurones were identified according to the bursts of spontaneous activity and to antidromic invasion established in repeated collision tests. The cells encountered in penetrations through the hippocampal formation at a depth ranging from 100 to 1400 μm below the surface, 300 μm lateral from the midline, were considered to be hippocampal cells. The position of the recording electrode track was verified histologically.

The effect of AVP on the spontaneous activity of single units was studied on 50 LSC and 27 hippocampal neurones. During iontophoretic application of AVP, 48% of the neurones in the lateral septum were excited (i.e. at least a 50% increase of the spontaneous activity), while 67% of the neurones in the hippocampus showed excitation. In the remaining neurones, the peptide-induced increase in spontaneous activity was either less than 50% or the activity remained unchanged. A decrease in activity during application of AVP was never observed. OXT induced excitation in 58% of the LSC cells (26 of 45) and in 61% of the hippocampal neurones (17 of 28). The remaining neurones in these structures were unaffected by OXT.

When Na^+ or Cl^- ions were ejected, as controls, through a barrel filled with 165 mM NaCl with current pulses of the same polarity and magnitude as used for the

ejection of AVP or OXT, the excitations induced by the peptides were never reproduced.

In more than 90% of the neurones tested, the peptide-induced excitations started shortly after the onset of the iontophoretic administration, reached a 'plateau' within 3–5 sec and remained at this level until the end of the ejection. With this short latency in onset and offset, the peptide-induced responses resembled the excitations elicited in LSC (see example in Fig. 1) and hippocampal neurones by glutamate. However, the peptide-induced responses differed from the Glu-evoked responses in at least one aspect. Increasing the Glu-expelling current frequently led to a decrease in the extracellularly recorded spike amplitude and firing rate of neurones, presumably as a result of 'depolarization block' [4]. In all but two neurones, this decrease in amplitude was never observed during application of AVP or OXT even when the peptides were ejected with the maximal current (200 nA) for at least 3 min (not shown). When AVP and OXT were tested on the same neurones, approximately one-third (n = 13) of the neurones responded to both peptides with excitation. Twenty of the remaining neurones were excited by either AVP or OXT. The mean expelling currents (± S.E.M.) necessary to excite orthodromically activated LSC neurones were similar for AVP and OXT (resp. 152.8 ± 1.4 and 117.1 ± 17.7 nA). Only few of the medial septal neurones responded to AVP or OXT with an increase of spontaneous activity. Only 3 of 18 MS cells could be excited by AVP. OXT excited 6 of 23 MS neurones tested.

In 10 of 18 LSC cells and 7 of 11 hippocampal neurones the response to Glu (ejected with 10 sec pulses of fixed current intensity delivered at ca. 30 sec intervals) was increased by at least 50% during iontophoretic application of AVP (see example

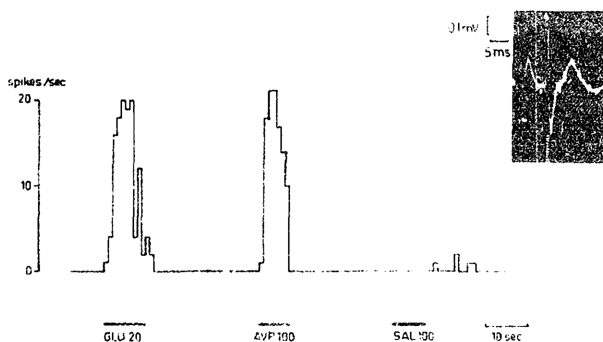

Fig. 1. The effect of iontophoretic application of glutamate (GLU, – 20 nA), vasopressin (AVP, – 100 nA) and 165 ml NaCl (SAL, – 100 nA) on the spontaneous activity of a lateral septal neurone. Horizontal bars show the duration of administration. The ordinate represents the number of spikes/sec. Inset: three superimposed oscilloscope sweeps displaying the orthodromic response of the neurone to stimulation of the fimbria-fornix fibres (lower tracing indicates the stimulus pulse).

in Fig. 2). OXT enhanced the Glu-induced responses in 8 LSC and 4 hippocampal neurones; the peptide was ineffective in two LSC cells and in one hippocampal neurone. The Glu-evoked responses of medial septal neurones were seldom increased during the application of AVP (1 of 9 cells) or OXT (1 of 8 cells).

Iontophoretically applied AVP and OXT affected many of the LSC and hippocampal neurones in a similar fashion and could induce excitations in these cells. This excitatory action could not have been due to current effects or to the action of the solvent ions, since appropriate balancing current was applied automatically during all iontophoretic tests and the peptide-induced responses could not be mimicked by ejection of Na^+ or Cl^- ions with the same currents as were used to expel the peptides. Iontophoretically applied AVP has been shown to excite lateral septal neurones [8], locus coeruleus neurones [14] and hippocampal pyramidal cells [17] in a similar way. Neuronal excitation following the iontophoretic release of OXT has only been shown in paraventricular neurosecretory cells [13]. In our experiments, AVP and OXT could still activate LSC neurones in which the spontaneous activity had been temporarily abolished by iontophoretically administered Mg^{2+} ions (preliminary observation). This indicates that the peptide-induced excitations must have been at least partly due to a postsynaptic effect. The AVP- and OXT-induced excitation of LSC and hippocampal neurones had a short latency in onset and offset, features resembling the action of a neurotransmitter [10]. However, it cannot be decided on account of the present experiments whether the short offset latency of the peptide-induced responses was due to a metabolic inactivation of the peptides or to fast diffusion.

A markedly smaller number of MS neurones than of LSC cells responded to microiontophoretically administered AVP and OXT. The low incidence of peptide sensitive cells among the antidromically activated neurones suggests that the cells

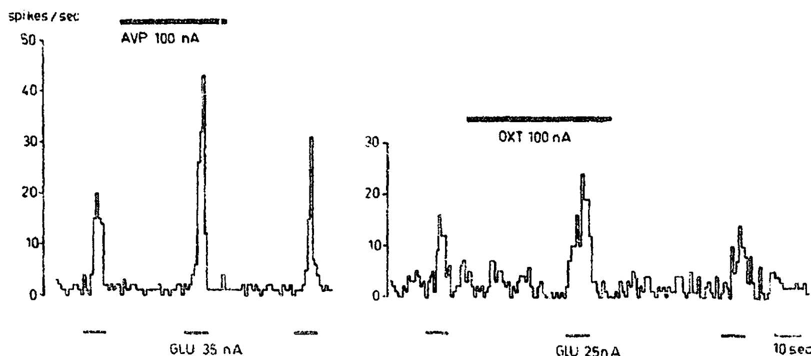

Fig. 2. Left: the effect of vasopressin (AVP, − 100 nA) on the responses of a hippocampal neurone to glutamate (GLU, − 35 nA). Right: the effect of oxytocin (OXT, − 100 nA) on the glutamate-induced excitation (GLU, − 25 nA) in a lateral septal neurone.

which project upon the hippocampus are less frequently the target for peptide action than the neurones which receive projection from the hippocampus. This is also suggested from the intraseptal distribution of peptidergic fibres. The highest density of these fibres was found in the LSC whereas only few fibres reacting with the AVP and OXT antisera were seen in the medial septal area [15].

The response of LSC and hippocampal neurones to Glu was increased during concomitant release of AVP or OXT. This action might not have been a mere summation of the excitatory effects of the peptide and the amino acid, since AVP and OXT frequently enhanced the Glu-induced response in neurones not excited by the peptides alone administered with the same or higher currents. There is increasing evidence that Glu, or a closely related excitatory amino acid, functions as neurotransmitter in the fimbria-fornix fibres innervating the LSC neurones. Both AVP and OXT could enhance the action of Glu, a potent natural agonist of the excitatory amino acid receptors [12, 20] on the LSC cells. This enhancement of amino acid neurotransmission may therefore represent another mechanism for the modulation of neural processes by AVP, OXT and closely related neuropeptides.

The investigations were supported by the Foundation for Medical Research FUNGO (grant no. 13-31-045, to I.J.A.U.).

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