Neuronal actions of oxytocin on the subfornical organ of male rats

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Department of Physiology, School of Allied Health Sciences, Osaka University Faculty of Medicine, Suita, Osaka 565-0871, Japan; and Max-Planck-Institut für Physiologische und Klinische Forschung, W. G. Kerckhoff-Institut, D-61231 Bad Nauheim, Germany

Hosono, Takayoshi, Herbert A. Schmid, Kazuyuki Kanosue, and Eckhart Simon. Neuronal actions of oxytocin on the subfornical organ of male rats. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E1004–E1008, 1999.—The aim of this study was to investigate effects of oxytocin (OT) on electrical neuronal activities in rat subfornical organ (SFO) and compare its action with the well-described excitatory effects of blood-borne angiotensin II (ANG II) on the same SFO neurons. With the use of extracellular recordings from spontaneously active neurons in slice preparations of the SFO, SFO neurons, 11.7% of tested neurons (n = 206) were excited and 9.7% were inhibited by superfusion with 10−6 M OT. Both excitatory and inhibitory effects of OT were dose dependent with similar threshold concentrations and were blocked by a specific OT-receptor antagonist but not by a vasopressin receptor antagonist. Blocking synaptic transmission with low calcium medium suppressed only inhibitory effects of OT. All but one of the OT-sensitive neurons were also excited by superfusion with ANG II at a concentration much lower than required for OT, suggesting that synaptically released OT rather than blood-borne OT alters the activity of SFO neurons in vivo. The results support the hypothesis that neurally released OT may modulate SFO-mediated functions by acting on OT-sensitive neurons.

angiotensin II; vasopressin; osmoregulation; extracellular recording

THE SUBFORNICAL ORGAN (SFO) is one of the circumventricular organs lacking the blood-brain barrier and has been implicated as a key structure for osmoregulation, especially in the induction of thirst elicited by circulating angiotensin II (ANG II). Direct injection of ANG II into the SFO causes thirst and salt appetite in rats, and these responses are absent after the SFO is lesioned (25). Electrical stimulation of the SFO also induces thirst (26) and causes the release of arginine vasopres- sin (AVP) and oxytocin (OT) from the neurohypophysis (27). Additional functions of the SFO have been suggested recently by showing that calcitonin, a peptide primarily involved in calcium homeostasis, strongly activates SFO neurons (23). These SFO-mediated functions are differently modified by many blood-borne peptides as well as by brain intrinsic hormones released locally as neurotransmitters (1, 8, 21, 24).

OT, which in the periphery facilitates milk letdown and stimulates uterine contractions in females, affects various regulatory behaviors that are centrally mediated in both sexes (18). As for dipsogenic behavior, intracerebroventricular injection of OT has been shown to inhibit water intake (2). Such injections also reduce salt appetite induced by ANG II (5) in both male and female animals (4), and treatments that inhibit salt appetite, such as hyperosmolality, stimulate central and pituitary release of OT (27). However, the exact sites of OT action for these responses are not yet clarified (12). The fact that oxytocinergic fibers are found in the SFO (7) suggests that OT may affect salt and water intake by acting on the SFO. The aim of this study is to investigate the effects of OT in comparison with those of ANG II on identical neurons of the rat SFO as a first step to clarify whether OT might affect the SFO-mediated functions at this level.

METHODS

In this experiment, 65 adult male Wistar rats (190–250 g) were used to avoid possible effects of estrous cycles in females. The materials and methods are the same, with minor modifications, as previously described (23). Briefly, rats were decapitated, and their brains were quickly removed and superfused with ice-cold artificial cerebrospinal fluid (aCSF). The brain was trimmed to a square block containing the entire hypothalamus. A slice of the body of the fornix containing the entire SFO was cut by hand and preincubated in aCSF at 35°C for 2 h. The average thickness of the slices was ~500 μm. The slice was transferred to a gilded recording chamber made from brass and was fixed to its bottom with a small metal weight. It contained a fluid volume of 0.7 ml when perfused with aCSF. During recordings, the temperature in the chamber was kept constant at 37°C by means of a Peltier element. The chamber was perfused at a rate of 1.6 ml/min with aCSF. The SFO could be easily identified by its yellowish protrusion into the third ventricle and the blood vessels lining the organ on both sides. Extracellular recordings were made from SFO neurons with glass-coated platinum-iridium electrodes. The recorded action potentials were amplified and displayed on a storage oscilloscope (Gould, Valley View, OH) and were analyzed with custom-made software (Spike2, Cambridge Electronic Design, Cambridge, UK) on a personal computer after passing through a window discriminator (World Precision Instruments). Once a stable recording from a single neuron had been established, its responsiveness was tested by switching to the perfusion solution containing the drug under consideration. From the continuous recorded rate meter counts, the average discharge rate of each neuron was evaluated for 60 s before the stimulus. According to previous reports (1, 21), a neuron was considered sensitive to the applied substance if the average change of discharge rates during the entire response time was larger than ±20% and at least ±0.5 spikes/s. The aCSF was equilibrated with 95% O2-5% CO2 and had the following composition (in mM): 124...
decreased their firing rate by response to 10−6 M OT. The absolute values of discharge rates of the neurons excited or inhibited by OT were not significantly different. The average latency from OT application to the onset of the response was 68 ± 18 s and did not differ between excitatory and inhibitory responses.

The excitatory responses to OT persisted during perfusion of low Ca++ and high Mg++ solution to block synaptic transmission in each of five neurons tested (Fig. 2A). In contrast, the inhibitory responses to OT disappeared during synaptic blockade in four of five neurons tested (Fig. 2B). In the low calcium medium, the fraction of neurons in which the change in discharge rate in response to OT application did not exceed ±0.5 spikes/s (Fig. 2, insets) was significantly larger in OT-inhibited than OT-excited neurons (χ² = 6.667, P < 0.01). Synaptic blockade with the low calcium medium changed the spontaneous activity in 9 out of 10 neurons; spontaneous activity was increased in six (by 49 ± 19%) and decreased in three neurons (by −59 ± 29%). These increases or decreases in spontaneous activity were not correlated with an excitation or inhibition by OT.

Coapplication of OTR-A blocked both excitatory and inhibitory effects of OT in each of the 10 neurons tested, 5 of which were excited and 5 of which were inhibited by OT. In the presence of OTR-A 10-fold in excess of OT, the excitatory responses to OT were reduced from 1.2 ± 0.2 to 0.1 ± 0.1 spikes/s (P < 0.05). Likewise, the inhibitory response to OT was reduced from −1.6 ± 0.6 spikes/s to −0.3 ± 0.2 spikes/s during superfusion with OTR-A (P < 0.05). In 13 OT-sensitive neurons, 10 were also sensitive to AVP; four of them were excited (Fig. 3A) and three of them were inhibited by both OT and AVP.
AVP, one was excited by OT and inhibited by AVP, and two were inhibited by OT and excited by AVP (Fig. 3B).

Coapplications of MC 10-fold in excess of OT did not block the response to OT of five neurons tested; two were excited and three were inhibited. In five AVP-responsive neurons, OTR-A did not block the response, which was, however, blocked in each case by MC. Neither OTR-A nor MC altered spontaneous discharge rates. The coapplication of MC did not significantly change the responses to OT (1.1 ± 0.2 without and 0.8 ± 0.2 spikes/s with the MC coapplication). The coapplication of OTR-A was also without significant effect on the responses to AVP (1.8 ± 0.5 without and 1.7 ± 0.7 spikes/s with the OTR-A coapplication).

Responsiveness to both ANG II and OT could be successfully tested in 82 neurons (Fig. 4). As in previous reports (23, 24), ANG II caused exclusively excitatory responses in 70.7% of all neurons tested. Table 1 shows that 13 (92.9%) of the 14 OT-sensitive neurons were also ANG II sensitive.

The proportion of OT-sensitive neurons was significantly higher in ANG II-sensitive neurons than in ANG II-insensitive neurons ($\chi^2 = 3.992, P < 0.05$).

**DISCUSSION**

This is the first study investigating the effects of OT on neuronal electrical activity in the rat SFO. In this study, 21.4% of all SFO neurons responded to $10^{-6}$ M OT, with similar fractions being excited and inhibited. Both excitatory and inhibitory responses were dose dependent and reversible with similar threshold concentrations and were blocked with a potent and selective OTR-A, which shows little affinity to AVP (17). The responses to OT were, however, not blocked by the potent AVP-receptor antagonist (MC). Therefore, the responses of SFO neurons to OT are mediated by OT-specific receptors. Functional evidence for OT receptors in the SFO has been previously provided by showing that application of OT on cultured SFO neurons increased intracellular Ca$^{2+}$ detected by fluorescence measurement with fura 2 dye (14). It was also
reported that OT-receptor mRNA is strongly distributed in the anteroventral third ventricle (AV3V) region of which SFO is a part, without, however, mentioning the SFO explicitly (30), although there are no autoradiographic studies in which OT-binding sites in the SFO have been explicitly reported (3).

In contrast to the uniformly excitatory action of ANG II on a large fraction of SFO neurons (21, 23), OT affected only a minority of SFO neurons exciting or inhibiting similar fractions. However, OT responsiveness was restricted almost exclusively to ANG II-responsive neurons, indicating that the OT receptors are not randomly distributed among the SFO neurons. The fact that only the inhibitory responses could be blocked by low Ca\(^{2+}\) and high Mg\(^{2+}\) solution suggests that the inhibitory responses were due to local inhibitory synaptic inputs from neurons excited by OT. In this respect, the action of OT resembled that of AVP (1). Although transmitters of the inhibitory synapses have not been determined in this study, \(\gamma\)-aminobutyric acid was reported to exist in SFO, according to histological evidence (20), and to cause inhibitory responses in SFO neurons, demonstrated by electrophysiological intracellular recordings (11). It has also been reported that nitric oxide synthase exists in the SFO and that nitric oxide acts as an inhibitory messenger in the SFO (21).

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In the present study, the threshold concentration of OT to induce clear responses of SFO neurons was below \(10^{-6}\) M. This coincides well with the OT responsiveness found in previous electrophysiological investigations with brain slices of the paraventricular nucleus (PVN) (15) and supraoptic nucleus (15, 29) of rats. The concentrations of OT required to elicit significant effects on neuronal activity seem to exclude the involvement of blood-borne OT acting on the SFO under physiological conditions, because peripheral OT levels are of the order of \(10^{-11}\) M even under conditions that stimulate OT secretion such as suckling, parturition, and high plasma osmolality (13, 28). Therefore, synaptically released OT might be much more relevant than circulating OT for affecting SFO neurons because the effective concentration may most likely be reached only in the synaptic cleft. Indeed a portion of oxytocinergic neurons that originate from the PVN and supraoptic nucleus terminates in the SFO (7, 16).

For ANG II with plasma concentrations higher than those of OT (10), the threshold concentration of responsive SFO neurons is \(10^{-9}\) M under the same experimental conditions (23). It is well documented that blood-borne ANG II directly activates SFO neurons and elicits thirst and other SFO-mediated functions (8, 9, 12). The fact that almost all OT-sensitive neurons in the SFO were also sensitive to ANG II may suggest that neurally released OT preferentially affects SFO-mediated functions of ANG II such as dipsogenic behavior and salt intake. Intracerebroventricular injections of OT have been reported to cause reduction of drinking and salt appetite (2, 4, 5). If the effects of OT on dipsogenic behavior are caused by OT actions on SFO neurons, the majority of OT-sensitive neurons in the SFO should elicit inhibitory responses to applied OT. However, OT excited as many neurons as it inhibited. The role of OT in the SFO, therefore, seems more complicated compared with that of ANG II, which causes excitatory responses exclusively. Because high-affinity OT-binding sites are distributed in drinking-related regions such as the AV3V region as well, those regions may also contribute to the effects of OT on drinking in vivo. To clarify the mechanisms for OT-induced dipsogenic behaviors, observations of the behavior with more localized OT applications in circum-

Table 1. Numbers of subfornical organ neurons responsive to ANG II and OT

<table>
<thead>
<tr>
<th>Response to ANG II</th>
<th>Excitation</th>
<th>Inhibition</th>
<th>No response</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation</td>
<td>7</td>
<td>6</td>
<td>45</td>
<td>58</td>
</tr>
<tr>
<td>Inhibition</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>No response</td>
<td>0</td>
<td>1</td>
<td>23</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>7</td>
<td>68</td>
<td>82</td>
</tr>
</tbody>
</table>

ANG II, angiotensin II (\(10^{-7}\) M); OT, oxytocin (\(10^{-6}\) M).
ventricular regions are required, together with electrophysiological studies on neurons of those regions. The SFO neurons responding to OT may also be a part of a neuronal feedback loop between the SFO and PVN because electrical stimulation of the SFO or local application of ANG II stimulates neuronal activity of PVN neurons and OT secretion (6, 8, 22). Lesions of the SFO or disconnections of SFO efferent fibers abolish the effect of the electrical stimulation of the SFO on OT secretion (6). A reciprocal neuronal circuit interconnecting SFO or disconnections of SFO efferent fibers abolish mechanisms of central integration. Front. Neuroendocrinol. 18: 292–353, 1997.


