Experimental Study

Serotonergic involvement in stress-induced vasopressin and oxytocin secretion

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Abstract

Objective: To investigate the involvement of serotonin (5-hydroxytryptamine – 5-HT) receptors in mediation of stress-induced arginine vasopressin (AVP) and oxytocin (OT) secretion in male rats.

Design: Experiments on laboratory rats with control groups.

Methods: Different stress paradigms were applied after pretreatment with intracerebroventricular infusion of saline or different 5-HT antagonists.

Results: Restraint stress (5 min), hypotensive hemorrhage or dehydration for 24 h increased AVP secretion fivefold and OT secretion threefold. Swim stress for 3 min had no effect on AVP secretion, but increased OT secretion threefold. Ether vapor or hypoglycemia had no effect on AVP or OT secretion. The restraint stress-induced AVP response was inhibited by pretreatment with the 5-HT2A+2C antagonists ketanserin (KET) and LY-53857 (LY) and the 5-HT3 antagonist ICS-205930 (ICS), whereas the 5-HT1A antagonist WAY-100635 (WAY) had no effect. The OT response to restraint stress was inhibited by WAY, KET and LY but not by ICS. KET and LY inhibited OT response to dehydration, and LY inhibited OT response to hemorrhage. Neither of the antagonists affected AVP responses to dehydration or hemorrhage, nor the swim stress-induced OT response. KET and LY inhibited OT response to dehydration, and LY inhibited OT response to hemorrhage. Neither of the antagonists affected AVP responses to dehydration or hemorrhage, nor the swim stress-induced OT response.

Conclusion: 5-HT2A, 5-HT2C and possibly 5-HT1A and 5-HT4 receptors, but not 5-HT1A receptors, are involved in the restraint stress-induced AVP secretion. 5-HT does not seem to be involved in the dehydration- or hemorrhage-induced AVP response. The restraint stress-induced OT response seems to be mediated via 5-HT2A, 5-HT2C and possibly 5-HT1A and 5-HT4 receptors, but not 5-HT1A receptors, are involved in the restraint stress-induced AVP secretion. 5-HT does not seem to be involved in the dehydration- or hemorrhage-induced AVP response. The restraint stress-induced OT response seems to be mediated via 5-HT2A, 5-HT2C and possibly 5-HT1A and 5-HT4 receptors. The dehydration and hemorrhage-induced OT responses are at least mediated by the 5-HT2A and 5-HT2C receptors. The 5-HT1A and 5-HT4 receptors are involved in stress-induced OT secretion.

European Journal of Endocrinology 147 815–824

Introduction

Arginine-vasopressin (AVP) and oxytocin (OT) are synthesized in the magnocellular neurons of the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN). Following axonal transport, AVP and OT are released from nerve terminals in the neurohypophysis upon stimulation. Various types of psychological or physical stressors have been shown to stimulate the hypothalamo–neurohypophysial system and to increase the secretion of AVP and OT (1–3). However, the relative release of AVP and OT depends on the specific stressor applied (4–6). Two decades ago it was considered that OT but not AVP was a stress hormone in the rat (7, 8). This has since been modified, but controversies about the AVP response to different stressors still exist. It has been proposed that primary physical stressors such as hypovolemia, hemorrhage, hypoglycemia or exercise increase AVP secretion (9, 10), whereas predominant psychological stressors such as restraint stress, ether-vapor stress or forced swimming have minor or no effect (6, 11–13). However, recent studies indicate that this is not necessarily true, since repeated psychological stress was found to increase the concentration of AVP in the PVN (14) or the median eminence (15) or gene expression of AVP in the PVN (16). Together, these findings indicate that both types of stress activate the hypothalamo–neurohypophysial system, which may not necessarily be reflected in elevated peripheral hormone levels (17, 18).

Several neurotransmitters seem to be involved in the mediation of the stress-induced release of AVP and OT, since administration of acetylcholinergic, histaminergic and aminergic receptor antagonists and administration of GABA and opiate receptor agonists inhibit the
neurohypophysial hormone response to various stressors (19–23). In addition, serotonin (5-hydroxytryptamine – 5-HT) seems to be involved in the mediation of the basal as well as the stress-induced secretion of the neurohypophysial hormones (24, 25). The effect of 5-HT on the secretion of the neurohypophysial hormones is exerted via centrally located receptors, of which primarily 5-HT2C receptors are known to be involved in the release of AVP, and 5-HT1A and 5-HT2 receptors in OT release (26–28). Recently, we reported that the 5-HT4 receptor might also be involved in the 5-HT-induced secretion of AVP and OT, whereas the 5-HT1 receptor seems to be of minor importance (29).

We have previously found that 5-HT1, 5-HT2 and 5-HT3 receptors are involved in stress-induced release of prolactin (30) and that 5-HT1A and 5-HT2C receptors seem to be involved in the stress-induced adrenocorticotropin (ACTH) secretion (31). Therefore, the aim of the present study was to investigate (1) which types of stress affect AVP and OT secretion, (2) whether the serotonergic system participates in the mediation of the release of the neurohypophysial hormones in response to different stress paradigms, and (3) which 5-HT receptors are involved in this response.

Materials and methods

Animal procedures

Male Wistar rats (250–325 g), bred at the Panum Institute, were used in all experiments. The rats were housed under controlled temperature (22 ± 1°C) and humidity (80%), in cages of four animals before and after surgery. A cycle of 12 h light: 12 h darkness with lights on at 0600 was used. The rats had free access to laboratory chow and tap water, when not dehydrated. The experimental protocol was in accordance with, and accepted by, the European Communities Council directive of 24 November 1986 and the Danish Ministry of Justice, Board for Animal Experimental Research. The animal experiments endeavored to reduce the overall number of animals and to reduce or eliminate all unnecessary stress or pain to the animals.

Drugs

The following 5-HT receptor antagonists were used: the 5-HT1A receptor antagonist WAY-100635, N-tert-butyl-3-(4-(2-methoxyphenyl) piperazine-1-yl)-2-phenylpropionamide (WAY), gift from Lundbeck Inc., Valby, Denmark; the 5-HT2A/+2C receptor antagonist ketanserin, 3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl]-2,4(1H,3H)-quinazolinedione tartrate (KET), RBI, Natick, MA; USA; the 5-HT2C(+/2A) receptor antagonist LY-53857, 6-methyl-1-[(-methyl ethyl)-ergoline-8β-carboxylic acid 2-hydroxy-1-methyl propyl ester malate (LY), RBI; and the 5-HT4 receptor antagonist ICS-205930, endo-8-methyl-8-axabicyclo [3.2.1]oct-3-ol indol-3-yl-carboxylate hydrochloride (ICS; tropisene), RBI. The receptor affinities of the antagonists are given in Table 1.

Insulin was purchased from Actrapid, Novo Nordisk, Bagsværden Denmark. All drugs were dissolved in saline and adjusted to pH 6.8–7.5, except for KET, which was dissolved in 0.9% saline acidified with 0.05% HCl and adjusted to pH 6.8 with 0.05 mol/l NaOH.

Experimental procedures

General procedures

For intracerebroventricular (i.c.v.) administration of 5-HT antagonists, a permanent stainless steel cannula was implanted in the right lateral cerebral ventricle through a drilled hole in the skull. The coordinates were: P = 0.0, L = 1.5, H = −4.0 relative to bregma. The cannula was closed with a platinum obturator. Compounds were infused intracerebroventricularly over a period of 2.5 min in a volume of 5 μl. All operations were performed during pentobarbital anesthesia (66 mg/kg i.p.) 4–5 days prior to the experiments. On the day of the experiments the animals were brought to the laboratory and allowed to de-stress in their cage 3 h prior to injections. After 90 min i.v. catheters and i.c.v. cannulas were extended by polypropylene plastic tubes permitting infusion of test substances without disturbing the rats. The antagonists

| Table 1 The 5-HT receptor antagonists used with the respective receptor affinities; values in pK₅ or pKᵦ. Shaded areas indicate the primary type of receptor specificity. A pK₅ above 7.0 is considered to indicate specificity for that receptor. Numbers in parentheses are reference numbers. |
|---|---|---|---|---|---|---|---|
| 5-HT1A | 5-HT1B | 5-HT2A | 5-HT2C | 5-HT3 | 5-HT4 | 5-HT5 |
| WAY (64) | 5-HT1A | 5-HT1B | 5-HT2C | 5-HT3 | 5-HT4 | 5-HT5 | 5-HT7 |
| 5-HT1A | 8.9 | <7.0 | <7 | <7 | <7 | – | – |
| KET | 5.9 (65) | 5.7 (65) | 8.7 (66) | 7.2 (66) | <4 (67) | – | 4.8 | 6.7 |
| ICS-205930 | 6.4 (68) | 5.5 (68) | 7.7 (66) | 8.3 (66) | – | – | – | – |
| 5-HT3+4 | 5.3 (68) | 4.6 (68) | 8.5 (67) | 6.2 (67) | – | – | – | – |

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were infused intracerebroventricularly at −15 min, unless otherwise stated. The time for administration and the doses of antagonists were chosen on the basis of previous experiments, with the antagonists administered both intraperitoneally and intracerebroventricularly (30, 32, 33).

In all experiments, blood samples were obtained by decapitation of rats at 0 min, which was scheduled between 1000 and 1200 h. Blood samples were collected in chilled polyethylene tubes containing 50 μl aprotinin (Trasylol, 20,000 kIU/ml; Bayer, Leverkusen, Germany) and 100 μl 0.5 mol/l EDTA and were kept on ice until centrifuged at 4°C. The plasma was separated into polyethylene tubes and stored at −20°C until hormone analysis could be carried out.

**Experiment 1. Effect of stress on AVP or OT secretion** Rats were exposed to one of the following seven different stressors. Restraint stress was performed by fixing the animal on its back in a supine position for 5 min. Cold-swim stress was performed by allowing the rat to swim in cold (4°C), deep water in a plastic bowl for 3 min followed by a 2-min period to allow it to shake the skin dry. Dehydration was performed for 24 h starting at noon. The length of dehydration chosen has previously been shown to cause significant AVP release (20) and did not lead to any visible discomfort for the animals. The Danish Government Animal Research Control Committee, Ministry of Justice, has approved animals. The Danish Government Animal Research (20) and did not lead to any visible discomfort for the animals.

**Hormone analysis** AVP and OT were analyzed by RIA in extracted plasma by means of C18 Sep-Pak cartridges as previously described (20). The least detectable quantity for AVP and OT was 0.1–0.3 pmol/l plasma and 4–6 pmol/l respectively. Intra- and interassay coefficients were 10 and 15% respectively for both assays.

**Osmolality analysis** Measurement of plasma osmolality was performed on plasma samples in triplicate by freezing-point depression (Advanced Microosmometer 3MO, Advanced Instruments Inc., Needham Heights, MA, USA) by a previously described method (34).

**Statistical tests**

Results are presented as the mean ± S.E.M. and evaluated by ANOVA followed by Newman–Keuls's test for multiple comparisons when appropriate. The level of significance was set at P < 0.05.
Results

Experiment 1. Effect of stress on AVP or OT secretion

Five minutes of restraint stress, 24 h of dehydration and 3.0 ml hemorrhage increased plasma AVP level six-, five- and fourfold respectively (P < 0.01, Fig. 1a), whereas swim, ether, endotoxin or hypoglycemia stress had no effect (Fig. 1a).

Restraint, cold-swim, dehydration and hemorrhage stress increased plasma OT level 3.5-, 2-, 3- and 2-fold respectively (P < 0.01, P < 0.05, P < 0.01 and P < 0.05 respectively, Fig. 1b), whereas ether or hypoglycemia stress had no effect (Fig. 1b).

Experiment 2. Effect of 5-HT antagonists on restraint stress-induced AVP or OT secretion

The restraint stress-induced AVP response was inhibited almost 70% by pretreatment with the 5-HT2A+2C antagonist KET, the 5-HT2C+2A antagonist LY and the 5-HT3+4 antagonist ICS (P < 0.01, Fig. 2a), whereas the 5-HT1A antagonist WAY had no effect (Fig. 2a). WAY inhibited the OT response to restraint stress almost 80% (P < 0.01; Fig. 2b). KET and LY reduced the OT response to restraint stress about 60% (P < 0.01, Fig. 2b), whereas ICS had no significant effect.

Experiment 3. Effect of 5-HT antagonists on dehydration-induced AVP or OT secretion

Twenty-four hours of dehydration increased plasma osmolality 4% from 288±1.2 mosmol/l to 300±1.2 mosmol/l (P < 0.05), AVP secretion twofold (P < 0.05), and OT secretion almost fourfold (P < 0.05) (Fig. 3a and 3b). Infusion of 5-HT antagonists had no effect on the dehydration induced AVP response (Fig. 3a). However, continuous i.c.v. infusion of the 5-HT2C+2A antagonist LY or the 5-HT2A antagonist KET inhibited the OT response 75% (P < 0.01, Fig. 3b), whereas the 5-HT3+4 antagonist ICS had no effect.

Figure 1 Effect of different stressors on plasma level of AVP (a) or OT (b). Restraint stress (RS; 5 min), dehydration (Dehy; 24 h), hypotensive hemorrhage (Hemo; 3.0 ml at 10 min), cold swim (Swim; 3 min in 4°C cold water), ether stress (Ether; 5 min) or hypoglycemia (Hypo; i.p. 3IU insulin at 45 min). Rats were decapitated at 0 min. Data represent means ± S.E.M. of eight rats. ## P < 0.01 and # P < 0.05 compared with control rats.

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Experiment 4. Effect of 5-HT antagonists on hemorrhage-induced AVP or OT secretion

Withdrawal of 3.0 ml of blood from the jugular vein increased AVP and OT secretion seven- and twofold respectively ($P < 0.05$, Fig. 4a and $P < 0.01$, Fig. 4b, respectively). Pretreatment with the 5-HT antagonist had no effect on hemorrhage-induced AVP secretion. OT secretion was inhibited 50% by LY, while ICS had no effect ($P < 0.05$, Fig. 4b).

Experiment 5. Effect of 5-HT antagonists on AVP or OT secretion induced by cold-swim stress

Three minutes of cold-swim stress had no effect on AVP secretion, but increased OT secretion twofold (Table 2, Fig. 1). None of the 5-HT antagonists affected this response (Table 2).

Discussion

The present experiments show that physical as well as psychological stress paradigms stimulate the secretion of the neurohypophysial hormones AVP and OT, since

Table 2 Effect of i.c.v. infusion of different 5-HT antagonists on the AVP and OT response to 3 min of cold-swim stress from $-5$ to $-2$ min, and decapitation at 0 min. Values are in pmol/l expressed as means of seven or eight rats ± S.E.M. *$P < 0.05$ versus control.

<table>
<thead>
<tr>
<th>Stress</th>
<th>Antagonist</th>
<th>AVP</th>
<th>OT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Saline</td>
<td>1.9±0.2</td>
<td>11±1.0</td>
</tr>
<tr>
<td>Swim</td>
<td>Saline</td>
<td>1.5±0.2</td>
<td>19±2.1*</td>
</tr>
<tr>
<td>Swim</td>
<td>WAY (5-HT1A)</td>
<td>1.5±0.1</td>
<td>23±2.3*</td>
</tr>
<tr>
<td>Swim</td>
<td>KET (5-HT2A)</td>
<td>1.9±0.2</td>
<td>21±1.7*</td>
</tr>
<tr>
<td>Swim</td>
<td>LY (5-HT2C)</td>
<td>1.8±0.1</td>
<td>26±1.5*</td>
</tr>
<tr>
<td>Swim</td>
<td>ICS (5-HT3)</td>
<td>2.4±0.2</td>
<td>25±3*</td>
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dehydration and hemorrhage (physical stressors), as well as restraint stress (psychological stressor) increased the secretion of both hormones. However, some of the applied stress paradigms, such as ether and hypoglycemia, did not affect the secretion of either of the two hormones. The stress response is probably an interaction between the neuroendocrine system, the sympathetic nervous system and the target organs, resulting in activation of different neuronal pathways and release of specific hormones (35).

Stress in itself releases various neurotransmitters, which may have either stimulating (catecholamines, 5-HT, histamine) or inhibiting (GABA) effect on hypothalamic and pituitary hormone release (19). The secretion of the two neurohypophysial hormones seem to be differentially affected by stress, since cold-swim stress, which is considered to be combined physical and psychological stressor, stimulated OT secretion, but not AVP secretion. The independent regulation of the neurohypophysial hormones illustrates the flexibility of the hypothalamo–neurohypophysial system, which is suitable to achieve a maximal overall positive effect on the target organs under different physiological conditions, such as bleeding, labor, hypovolemia, etc. (5). A heterogeneous hormone response in respect to AVP and OT has also been found after immobilization, forced swimming, ether stress, hemorrhage, endotoxin and osmotic loading (4–6, 36).

The present experiments show that involvement of 5-HT and the effect of 5-HT receptor blockade on the AVP or OT responses to different stress paradigms are not general, but seem to be as important as the involvement of other neurotransmitters, such as nor-epinephrine and histamine (20, 37, 38). The present finding of an increased level of plasma AVP after 5 min of restraint stress is in accordance with two studies (5, 7), but in contrast to the a previous finding, probably due to a short period of stress (1 min) in that

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**Figure 3** Effect of continuous i.c.v. infusion (0.3 nmol/h) of saline, KET or LY (5-HT<sub>2A,2C</sub>) or ICS (5-HT<sub>3</sub>+) during a 24-h dehydration period on the AVP (a) and OT (b) response. Rats were decapitated at the end of the dehydration period (0 h). Data represent means ± S.E.M. of eight rats; ## P < 0.01 compared with control; **P < 0.01 compared with saline-treated dehydrated rats.
The stimulating effect of restraint stress on plasma OT is in agreement with our own and others previous findings (4, 39, 40). Therefore, it seems that both AVP and OT are stimulated by restraint stress. A single prolonged period of restraint stress (30–150 min) has been found to increase AVP mRNA in the medial parvocellular part but not in the magnocellular part of the PVN (41, 42). However, an increased synthesis of AVP in the parvocellular PVN does not inevitably induce an increased plasma level of AVP in the peripheral circulation, since AVP produced in the parvocellular part of the PVN is released primarily into the pituitary portal circulation. The pattern emerging from the previous and the present findings indicates that single ultra short-term restraint stress (1–2 min) performed by mechanical fixation or restraint in a plastic glass box, does not seem to be sufficient to increase either AVP mRNA in the hypothalamic PVN or AVP in peripheral plasma (36, 43), whereas a 3–5 min period of manual restraint, as used in this experiment, is sufficient to increase peripheral plasma levels of AVP (5, 7).

Serotonergic antagonists with affinity for 5-HT2, 5-HT3 and 5-HT4 receptors inhibit the AVP response to restraint stress, whereas 5-HT1A and 5-HT2 antagonists reduce the OT response to this stressor. There are no available comparable data, but in unstressed rats, the AVP response to serotonergic stimulation is mediated via 5-HT2A and 5-HT3 receptors (26, 28, 44, 45), while 5-HT1A and 5-HT2 receptors are involved in the 5-HT-induced OT response via the PVN (46–49).

We found that dehydration is a potent physiological stimulus for both AVP and OT secretion, which confirms previous findings (9, 50). An involvement of the serotonergic system in AVP response to dehydration is possible but does not seem likely, since none of the antagonists (5-HT2A, 5-HT2C, 5-HT3, 5-HT4) used in the present experiments had any effect on this response. An
involvement of other receptors cannot be excluded. The possibility that the receptor blockade was insufficient is limited, since we used continuous i.c.v. infusion of compounds via an osmotic pump. In addition, using an identical experimental design, we previously found that the AVP response to dehydration was inhibited by histamine receptor antagonists (20). Furthermore, an increase in AVP secretion, induced by osmotic challenge with 500 mmol NaCl/l, was not affected by either the 5-HT2 receptor antagonist ritanserin or the 5-HT2A/C antagonist DOI (51).

Hypotensive hemorrhage-induced by acute depletion of approximately 20% of the total blood volume potently increased AVP and OT in peripheral plasma in our study. This is in accordance with previous findings, which showed that a 15–30% acute reduction in blood volume increased AVP (9, 52–54), and OT concentration in peripheral (5, 50, 55) and pituitary portal plasma (56), and increased AVP content and mRNA in the hypothalamus (52, 57, 58). The OT response to hemorrhage seems to be mediated via 5-HT2 receptors, since the response was inhibited by pretreatment with the 5-HT2 antagonist LY. The AVP response also tended to be inhibited, but this was not significant.

Cold-swim stress stimulated OT but not AVP secretion, which is in agreement with previous observations (6, 59). In the present experiments, rats were exposed to deep cold water (4°C for 2 min). The increase in OT secretion could be due either to the immersion in cold water or the swimming exercise, or both. Exposure to cold environment (4°C for 30 min) has previously been found to increase OT levels (5), and single or repeated forced swim stress (20°C for 10 min) increased both AVP and OT in microdialysates from both the SON and the PVN, but only OT in peripheral plasma (59). The OT response induced by cold-swim stress does not seem to involve the investigated 5-HT receptors.

Five minutes of ether stress did not affect AVP or OT secretion, which conflicts with previous findings both of ourselves and of others (4, 5, 36, 39). A reason for the missing response to ether stress may be the stress-time schedule, since it was found that 1 min of ether stress increased AVP or OT secretion twofold, measured 1 min after the end of the stress period, whereas the hormone response disappeared 4 min after the stress period was discontinued (4). In accordance with most of the previous studies (5, 39), we found no effect on insulin-induced hypoglycemia stress on AVP or OT secretion.

The present findings demonstrate that 5-HT is differentially involved in the stress-induced secretion of AVP and OT. One explanation might be that various stress paradigms activate cortical, limbic and hypothalamic areas differently, and these areas in addition have a heterogeneous distribution of 5-HT neurons. 5-HT nerve fibers are localized differently within the hypothalamus.

Although relatively few in the PVN, they are more abundant in the parvocellular part than in the magnocellular part and are primarily projecting to OT neurons, whereas they are very few and scattered at AVP neurons in the PVN (60, 61). The few 5-HT fibers in the SON are found to be in close contact with AVP neurons, but sparse at the OT neurons (62).

The involvement of different serotonergic receptors in the mediation of the stress-induced AVP and OT responses is found to be differentiated for, for example, blockade of 5-HT1A receptors inhibited restraint stress-induced OT, but not AVP secretion. This finding is in accordance with previous findings of a different involvement of 5-HT receptors in AVP and OT secretion (28, 63). The reason for this dissimilarity may be absence of 5-HT1A receptors on AVP neurons. The present study has not investigated the specific localization of the interaction between the 5-HT system and the neurohypophysial system. Further studies, for example, with specific anatomical lesions, are required to elucidate this.

In conclusion, we found that various stress paradigms affect AVP and OT secretion differently and that the serotonergic system is involved differently in these responses. The restraint stress-induced AVP and OT responses are at least mediated through 5-HT2A/C and 5-HT4 receptors, and via 5-HT1A and 5-HT2A/C receptors respectively. The dehydration- and hemorrhage-induced OT response is in part mediated through the 5-HT2A/C receptors, whereas the AVP responses to these stressors do not seem to involve the investigated 5-HT receptors. Swim stress increased only OT secretion, but the investigated 5-HT receptors are not involved in this response. Either and hypoglycemia stress had no effect on AVP or OT secretion.

Acknowledgements

This study was supported by grants from the Danish Medical Research Council: NOVO’s Foundation; The Lundbeck Foundation; Nordic Insulin Foundation Committee; The Danish Hospital Foundation for Medical Research, Regions of Copenhagen, The Faroe Islands and Greenland; and the Danish Medical Association Research Fund. We thank Elsa Larsen and Jytte Oxbøll for skilled technical assistance. The materials for RIA of AVP and OT were kindly provided by the National Hormone and Pituitary Program of the NIDDK.

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