THE RELEASE OF OXYTOCIN AND VASOPRESSIN IN THE NEUROINTERMEDIATE LOBE OF THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR) AND THE WISTAR-KYOTO RAT (WKY)

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SUMMARY

The release of arginine-vasopressin (AVP) and oxytocin (OT) from the rat neurointermediate lobe (NIL) of the pituitary was studied in vitro in the male spontaneously hypertensive rat (SHR) and the Wistar-Kyoto rat (WKY). The basal and stimulated (50 mM KCl; a submaximal dose) release of AVP were significantly higher in the SHR than in the WKY. No corresponding strain difference was seen in OT release. In the WKY, depolarisation released more OT than AVP. The GABA-A agonist muscimol attenuated the KCl induced release of both peptides. The findings indicate that a lower dose of muscimol is required to inhibit the AVP than the OT release. It is concluded that the strain difference in AVP release does not apply to OT terminals, which indicates a difference in mechanisms regulating the AVP and OT release at the NIL level.

KEYWORDS: Neurointermediate lobe, vasopressin release, oxytocin release, muscimol, GABA, spontaneously hypertensive rat.

INTRODUCTION

Arginine-vasopressin (AVP) and oxytocin (OT) are two structurally related peptide hormones released from the neural lobe that are involved in numerous physiological processes. A variety of stimuli can simultaneously or independently activate their release (10). In the adult male spontaneously hypertensive rat (SHR) the AVP mRNA in hypothalamic areas, the AVP content of the pituitary and the AVP level in plasma are increased compared to its normotensive counterpart, the Wistar-Kyoto rat (WKY) (12,16). Taken together this suggest an increased turn over of AVP in the hypothalamo-neurohypophyseal axis in the SHR. In contrast to AVP, the OT mRNA in corresponding

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hypothalamic areas and the OT content of the pituitary are decreased in the SHR compared to the WKY (16). As to OT plasma levels, conclusive differences have not been demonstrated. Hypothalamic content of both AVP and OT are decreased in the SHR. Apparently there is a difference between the two neuropeptides in the SHR vs WKY associated to the hypothalamo-neurohypophyseal axis, which might have a functional significance. AVP and OT are released by potassium induced depolarisation from isolated preparations of the neurointermediate lobe of the pituitary (NIL) (11,13). The basal as well as KCl stimulated AVP release is higher in the SHR than in the WKY (11). Analogous studies concerning OT have not been carried out. The high AVP release is an unique feature of the SHR. The release of AVP is about the same in WKY, Sprague-Dawley and Wistar male rats [11]. Besides the regulation of AVP and OT release at the level of the hypothalamus, their also exist a regulation in the pituitary (2). In the posterior pituitary the modulation of release may occur either directly by substances released nearby or indirectly by intermediating pituicytes (2). GABA (γ-aminobutyric acid) neurons terminate synaptically on pituicytes and magnocellular fibres, containing large dense core vesicles (3), and GABA-A binding sites have been demonstrated (1). Evidence is provided (11,13), although not totally unanimous (8), for a GABA inhibition of the release of AVP and OT from the NIL.

The aim of the present study was to examine and compare the release of OT and AVP from the NIL in the SHR and WKY in order to investigate if the differences between the two strains in AVP release (11) include OT as well. A dissimilarity between the AVP and OT release in this respect would suggest a difference in mechanisms regulating the AVP and OT release at the NIL level. The basal and the stimulated release were investigated as well as the inhibition achieved by muscimol.

MATERIALS AND METHODS

Male SHR (n=24) and WKY (n=24) rats (Möllegaard, Denmark), 13-14 weeks old, were used. They were kept under controlled conditions in an air-conditioned room at a temperature of 21±1°C and a 12 hour reversed day-night light cycle (light on at 2100 h and off at 0900 h). They had free access to commercial food pellets (R36; Lactamine, Södertälje, Sweden) and tap water. The NIL was rapidly taken out after decapitation, rinsed in normal Locke solution (NLocke; 140mM NaCl, 5 mM $KHCO_3$, 1 mM $MgCl_2$, 2.2 mM $CaCl_2$, 10 mM glucose, 10 mM hepes, pH 7.2) and placed between two nylon gauze mesh in a perfusion chamber continuously perfused with oxygenated (93.5 % O_2 + 6.5 % CO_2) NLocke for 45 minutes, at a rate of 0.5 ml/min and 37°C. This was followed by perfusion with 40 Na Locke (40 mM NaCl, 100 mM N-methyl-D-glucamine (NMDG); a sodium reduced buffer) for 15 minutes. Thereafter fractionation started (5min/fraction), while perfusion with 40 Na Locke continued for a total time of 240 min. The NIL was stimulated for 2 minutes at three occations (S_1=9, S_2=99 and S_3=189 min.) with K Locke (50 mM KCl, 40 mM NaCl, 50 mM NMDG or 100 mM KCl, 40 mM NaCl, 0 mM NMDG). The sodium reduced buffer was used to keep the osmolarity constant and the buffer inert when stimulating with 50 mM KCl and subsequently the sodium was

replaced with equivalent moles of NMDG. The stimulated release is the amount (fmol/ml) of peptide released after subtraction of the basal release. The effect of muscimol (Sigma, St Louis, MO) was studied at stimulation S_2 and S_3 (doses; 3 μM and 1 μM respectively) by adding muscimol to the 40 Na Locke 30 minutes before and after the stimulations (S_2 and S_3) and also to the K Locke. Control experiments were run in parallel using identical procedures but the muscimol administration was excluded. The ability of muscimol to inhibit the KCl induced release of AVP and OT is expressed as the ratio between the first and the second stimulation (S_2/S_1; S_1=KCl, S_2=KCl with or without 3 μM muscimol) and the first and third stimulation (S_3/S_1; S_1=KCl, S_3=KCl with or without 1 μM muscimol). This expresses the fraction of released peptide compared to S_1. The radioimmunoassays of AVP and OT were performed as previously described (9,11). AVP, OT and AVP-antiserum A8 were provided by Ferring, Sweden and OT-antiserum RI3 by the Division of Neurophysiology and Neuropharmacology, MRC, London. AVP and OT were monoiodinated according to the chloramine-T procedure (4).

RESULTS

The potassium chloride stimulated release of AVP and OT was studied at two different KCl concentrations (Table I). Both strains significantly increased the release of AVP and OT about five to eight times after doubling the KCl concentration. The concentration 50 mM KCl was used in the following experiments.

Table I. The relationship between KCl concentration and the stimulated release of vasopressin and oxytocin.

Strain	KCl (mM)	Vasopressin (fmol/ml)		Oxytocin (fmol/ml)	
SHR	50	2900± 895	(4)	2410 ± 370	(4)
	100	21300 ± 1090	(4)†*	19200 ± 1210	(4)*
WKY	50	1280 ± 433	(3)	4140 ± 1340	(3)
	100	11100 ± 1710	(4)*	22800 ± 2620	(4)*

The values are means ± SEM(n). Statistical significances (Mann-Whitney U-test): 100 mM vs 50 mM: * $p<0.05$; SHR vs WKY: † $p<0.05$.

The basal release of AVP was significantly higher in the SHR than in the WKY (Table II). No corresponding difference in basal release of OT was seen. When stimulated with 50 mM KCl, about twice as much AVP was released in the SHR compared to WKY. In contrast no inter-strain difference

was achieved in stimulated release of OT. A comparison between the amount released of the two peptides reveals that in the WKY a depolarisation released more OT than AVP (Table II).
No significant correlation appeared between the release of AVP and OT (Pearson rank correlation; SHR: r=0.48, p=0.23 and WKY: r=0.55, p=0.10).

Table II. Basal and stimulated release (50 mM KCl) of vasopressin and oxytocin.

Strain	Basal release (fmol/ml)		Stimulated release (fmol/ml)	
	Vasopressin	Oxytocin	Vasopressin	Oxytocin
SHR	97.7 ± 18.5 (23)*	113 ± 20.6 (23)	2210 ± 324 (19)**	1750 ± 232 (19)
WKY	47.8 ± 7.9 (23)	108 ± 19.7 (23)	844 ± 116 (19)††	1930 ± 375 (19)

The values are means ± SEM(n). Statistical significances (independent t-test): SHR vs WKY * $p<0.05$, ** $p<0.01$ and (dependent t-test): AVP vs OT †† $p<0.01$.

Muscimol (3 μM) significantly inhibited the KCl stimulated release of AVP (Table III), with no difference between the strains. The lower dose of muscimol (1 μM) caused a significant decrease of AVP in the WKY group but not in the SHR. The effect of muscimol (3μM) on the OT release reached statistical significance only in the SHR. In the WKY the stimulated OT release after muscimol treatment was not different from that of controls.

DISCUSSION

The data confirms our previous observations that the basal and stimulated release of AVP from the NIL were amplified in the SHR compared to the WKY (11). In the present study we detected no corresponding strain difference in the OT release. The release was tested at two dose levels of KCl to make sure that a difference between the two strains was not hidden by a maximal OT release already at 50 mM KCl. However, the release of OT was about 5–8 times higher after 100 mM KCl in both strains than after 50 mM KCl. We therefore suggest that the increased release of AVP in the SHR vs WKY is specific to AVP and that the mechanism which is the basis for this strain difference does not include the release of the closely related peptide OT. The fact that no interstrain differences existed in OT release indicates that the augmented NIL AVP release in the SHR is unlikely to be due to general neurophysiological mechanisms such as altered electrical properties and calcium handling.

AVP and OT are localized in separate neurons in the posterior pituitary (14) and its release is influenced in a dissimilar way by e.g. opioid peptides and autofeedback mechanisms (2,5–7,15). The present data support the existence of different regulatory mechanisms for the release of AVP and OT. The utilisation of the isolated NIL preparation demonstrates that differences exist at the level of the NIL. Which factor that would be responsible for the augmented AVP release in the SHR is, however, not possible to state at present.

Table III. The effect of muscimol treatment on stimulated release of vasopressin and oxytocin.

Animals	Muscimol (μM)	Ratio[a)]	
		Vasopressin	Oxytocin
SHR	3	0.67 ± 0.11 (7)**	0.59 ± 0.08 (6)*
WKY	3	0.60 ± 0.08 (8)**†	0.93 ± 0.13 (8)
SHR, controls	-	1.12 ± 0.11 (8)	0.93 ± 0.13 (8)
WKY, controls	-	1.10 ± 0.15 (7)	1.17 ± 0.08 (7)
SHR	1	0.82 ± 0.12 (6)	1.01 ± 0.25 (6)
WKY	1	0.81 ± 0.12 (8)*	1.16 ± 0.22 (7)
SHR, controls	-	1.19 ± 0.18 (8)	0.97 ± 0.14 (8)
WKY, controls	-	1.19 ± 0.12 (7)	1.21 ± 0.26 (7)

Values are means ± SEM(n). Statistical significances (independent t–test): muscimol vs controls, within strain comparison *$p \leq 0.05$, **$p \leq 0.01$ and (dependent t–test): AVP vs OT † $p \leq 0.05$. a) S_2/S_1 orS_3/S_1, for details see text.

We have earlier reported on altered GABA responsiveness in the NIL of the SHR (11). In the present study the GABA–A agonist muscimol was effective to inhibit the KCl induced release of

AVP. However, the dose of muscimol which was effective to decrease the release of AVP (3μM muscimol) in the two strains had only an effect on the OT release in the SHR and no significant effect was achieved in the WKY. Thus, the data indicate that in the WKY the regulation of OT release is less sensitive to muscimol than the mechanisms involved in the AVP release or might not be influenced by a GABA-A agonist treatment. Considering that more OT than AVP was released and that only the AVP release was influenced by the muscimol treatment in the WKY (Table II and III), the effect of muscimol might be related to the amount released, that is the more peptide that is released the higher dose of muscimol is required in order to inhibit the release.

It is concluded that in the SHR, compared to its normotensive counterpart the WKY, the depolarization induced release of AVP from the NIL is augmented. The observation that this difference does not include a corresponding increased release of OT in the SHR, indicates that partly different mechanisms regulate the AVP and OT secretion at the NIL level. The possibility that GABAergic mechanisms are involved in this difference has to be further elucidated.

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