Vasopressin and Oxytocin Release in the Brain - a Synaptic Event

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In order to obtain evidence for a central release of vasopressin and oxytocin, the release of these peptides was demonstrated in various extrahypothalamic areas of the rat brain. It proved that in those areas where these peptidergic fibers terminate synaptically a vasopressin and/or oxytocin calcium-dependent release, similar to that in the neurohypophysis, could be evoked by potassium or veratridine. Such release was not found in areas in which these fibers do not exhibit synaptic specialization.

INTRODUCTION

One of the criteria for establishing a substance as a neurotransmitter is its presence within synaptic structures and its release upon a depolarizing stimulus. Release after depolarization by high potassium or veratridine has been demonstrated for a variety of amine, amino acid and peptidergic (putative) neurotransmitters¹⁷. For arginine vasopressin (AVP) and oxytocin (OXT), two peptides produced in the paraventricular (PVN) and supraoptic (SON) nucleus, such a release has only been demonstrated in the neural lobe^{8,15,21}. In this neurohaemal contact zone the fibers from these nuclei terminate on the basal membrane of the blood vessels²⁰, so that AVP and OXT are released into the bloodstream and transported to their target structures as hormones. However, in addition to the extensive projections towards the neural lobe¹ these two nuclei reach many areas outside the hypothalamus^{3,6,22,26}, where their peptidergic fibers terminate synaptically on other neuronal structures⁵. These and other data led to the suggestion that these two classical neurohormones might function as neurotransmitters in the central nervous system^{4,5,23}.

In order to provide more evidence to support this hypothesis it was studied whether in these extrahypothalamic brain regions AVP and OXT might be released after a depolarizing stimulus. For this purpose, the lateral septum, being a main target area

for AVP fibers and the nucleus tractus solitarius (NTS) for OXT fibers³ was chosen. The results are compared with the AVP and OXT release from the neural lobe in the same experiment. As morphological data in the literature suggest that noradrenaline and dopamine might be released from varicosities that do not exhibit synaptic specializations^{2,9,10}, it was studied whether this might also be possible for AVP and OXT. Since the AVP and OXT containing fibers between the PVN and SON are varicose and fail to evince synaptic specializations (Buijs, unpublished results), this area was selected for this purpose.

MATERIALS AND METHODS

Male Wistar rats (160–180 g) were killed by decapitation. The septum and NTS tissue was dissected and chopped at intervals of ~ 0.3 mm in two directions, placed in an open incubation vial at room temperature in 1 ml oxygenated buffer (NaCl 154 mM; KCl 5.6 mM; CaCl₂ 2.2 mM; MgCl₂ 1 mM; Tris 2 mM; NaHCO₃ 6 mM; glucose 10 mM; 0.5 mg/ml bovine serum albumine, pH 7.4). After approximately 45 min, 9 vials were collected, each containing 3 septi, 3 NTS or 3 PVN/SON. Three further vials each contained a neurointermediate lobe. The samples were placed in an oxygenated Dubnof incubator at 37 °C, with continuous agitation. Except for the first incubations, which took 15 min, the in-

cubation fluid was changed at 10 min intervals. These 1 ml samples were collected in a tube containing 0.1 ml 1 N HCl, frozen immediately and stored at -20°C until extraction in the radioimmunoassay (RIA). AVP and OXT were measured simultaneously in the same sample, except for the septal region, in which only AVP was measured. Details on the extraction procedure and RIA for AVP and OXT have been described elsewhere^{12,13}. The AVP and OXT RIA had a negligible cross-reaction of 0.01% with OXT and AVP, respectively. The release was expressed as a percentage of the total amount of AVP or OXT. This value was calculated by adding the peptide released to the amount of peptide left in the tissue after incubation. If the values found in the medium were below the detection limit of the assay, the detection limit was used as an actual value.

RESULTS

Using the present incubation procedure basal levels were already reached after the first 15-min incubation period (Fig. 1A, B). The storage of the material in buffer at 0°C instead of 20°C until dissection of all samples was completed, resulted in a diminished stimulated release by potassium or veratridine of AVP and OXT, in neural lobe, septum or NTS (Fig. 2A, B). In addition, in the neural lobe the release of AVP at 0°C was 5 times higher than that at 20°C . No difference in release pattern could be observed if the material was kept at 37°C instead of 20°C until dissection was completed. Furthermore, the addition of bacitracin ($50\text{ }\mu\text{g/ml}$) did not affect the AVP and OXT levels.

In the neurointermediate lobe high potassium

Fig. 1. Release of vasopressin (A) and oxytocin (B) from isolated neurointermediate lobes. Note the differences in the percentage of, vasopressin and oxytocin released. The initial release during the dissection of the material is indicated in the hatched column. -Ca²⁺, no calcium in the medium; +K⁺, high potassium (56 mM); Ver, use of 50 μM veratridine in the incubation buffer. The detection limit of the assay is indicated by the horizontal dotted line. Mean $\pm$ S.E.M. is indicated. The total sum of the amounts present in the tissue after the experiment and the released peptide are indicated by the 100% value.

Fig. 2. The release of oxytocin from the nucleus tractus solitarius (NTS) after dissection at different temperatures. Dissection of the material at 0 °C (A) results especially during potassium stimulation in a diminished release as compared with the NTS dissected at 20 °C (B). The 100% value indicates the amount from 3 NTS. For legend to the figure see Fig. 1.

resulted in a tenfold rise in the release of OXT and AVP, whereas veratridine resulted in a 14-fold and 19-fold release of AVP and OXT, respectively (Fig. 1A, B). Throughout the experiment, absolutely, but also expressed as a percentage of the total, much more (169 ng = 21%) OXT than AVP (45 ng = 6%) was released, while the neural lobe content of both peptides is approximately equal.

AVP levels in the first sample (obtained during the 20 °C storage) from the septum and NTS were much higher than those of OXT in the same medium. The highest level was obtained from the NTS, where more than 25% of the tissue stores were released, while OXT release was little over 1%. In subsequent incubations in the NTS neither with potassium or veratridine stimulation, AVP release above detection level could be demonstrated, while the OXT-stimulated release pattern from the NTS

was comparable with that in the neural lobe.

Basal OXT or AVP release from NTS or septal slices, respectively, was too low to be measured accurately; thus the potassium-stimulated release in both regions was approximately 4 times the detection limit of the assay (Figs. 2B, 3). In all cases the omission of Ca²⁺ resulted in an inhibition of the potassium stimulated release. In a separate experiment it was found that Ca²⁺ was also necessary to obtain veratridine stimulated release.

In slices from the region between the PVN and SON the total content of AVP and OXT was much higher than that in the septum or NTS, viz. 20 and 10 ng, respectively; however, no potassium-stimulated release of AVP or OXT could be observed. Only a very small amount of OXT was released upon veratridine stimulation (Fig. 4A, B).

Fig. 3. Vasopressin release from septal slices. Veratridine stimulated release is not different from the potassium stimulated release. The 100% value indicates the amount of AVP from 3 septi. For legend to the figure see Fig. 1.

DISCUSSION

The pattern and amount of AVP and OXT released from the neural lobe as observed during this experiment, demonstrates the validity of the present procedure since the values obtained are well in line with those reported earlier^{8,15,16,21}. In addition, the amounts of peptide as recovered from the whole procedure are not different from those found when AVP and OXT were measured directly in extracts of neural lobe, septal area or NTS (refs. 12, 14 and Dogterom and Buijs, unpublished observations). It has already been demonstrated that the release of AVP from the neural lobe is increased at temperatures below 15 °C¹⁵, which explains the high AVP release from the neural lobe during the incubation at 0 °C. However, the different release pattern found in all

brain regions at 37 °C after tissue storage at 0 °C indicates that low temperatures induce, in addition, a change in the membrane properties in such a way that it cannot readily be restored by the subsequent incubation at 37 °C. Another possibility is that at such a low temperature the membrane potential is lowered so much that the incubation at 37 °C will take longer than 1 h to fully restore it.

The present study, in which the potassium- and veratridine-stimulated release from the neural lobe of both AVP and OXT could be compared, demonstrates that these peptides are released differentially, with a much more facilitated release of OXT than that of AVP. The ratio between the potassium and veratridine-stimulated release of OXT from the neural lobe vs that of AVP in the present in vitro study, is approximately 5, which is the same ratio as found for AVP/OXT in blood plasma¹¹. This suggests that, whereas the AVP and OXT levels in the neural lobe are almost equal, the amount of peptide released in the in vitro system is a reflection of the physiological conditions of the tissue at the moment of the dissection. However, whether this holds true for various different circumstances will have to be tested, e.g. in a series of animals in which the hypothalamo-neurohypophyseal system has been stimulated or inhibited.

The basal OXT or AVP release in NTS or septum is below the detection limit of the assay, therefore no indication can be given as to how much the stimulated release is above the actual basal release level. It appears, however, that the release patterns of AVP from the septum and OXT from the NTS, are comparable to the release patterns of these peptides from the neural lobe. The release of AVP in septum and neural lobe seems to be less sensitive to veratridine depolarization than that of oxytocin. The low vasopressin release evoked by veratridine in the septum is apparently not due to exhaustion of the tissue stores, since using veratridine before the potassium stimulation resulted — just as with potassium — in a 3.5% release of the tissue stores (unpublished results). The mechanism behind the difference in veratridine stimulated release of AVP and OXT is yet unknown but might indicate different membrane properties for the respective neurons and a different release mechanism of these peptides also in vivo.

The present experiment demonstrates that in

Fig. 4. The release of vasopressin (A) and oxytocin (B) from the region between the paraventricular (PVN) and supraoptic nucleus (SON). No potassium or veratridine stimulated release could be observed for vasopressin, while the veratridine evoked oxytocin release was only slightly higher than the basal release. $P < 0.05$. The 100% value indicates the amount from 3 PVN/SON. For legend to the figure see Fig. 1.

brain regions where AVP and OXT containing fibers terminate synaptically⁵, these peptides can be released upon a depolarizing potassium or veratridine stimulus. In addition, it has been demonstrated that this process is Ca²⁺ dependent. Herewith another aspect for these peptides has been shown in which they act as neurotransmitters. Thus, apart from their now reported release also the anatomical localization^{3,5,22,24}, electrophysiological data^{18,19} and degradation processes⁷ point to the neurotransmitter properties of these neurohypophyseal hormones in the central nervous system.

The present study also demonstrates that AVP and OXT containing fibers with many varicosities

but without synaptic specialization, do not release their peptidergic content upon a depolarizing stimulus, in spite of the high amounts of peptides in the tissue. Consequently, this result suggests that these peptides are only released synaptically or from fibers in contact with neurohaemal zones, and not from the varicosities without synaptic specializations that these fibers exhibit so conspicuously.

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