Distribution of Vasopressin and Oxytocin in Rat Brain

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Abstract

Arginine-vasopressin and oxytocin in various portions of rat brain were determined by radioimmunoassays. The hormones were extracted from tissue samples into 0.1N HCl and then purified partially with acetone-petroleum ether extraction. The non-equilibration method was used for the assays. In this method recovery rates of arginine-vasopressin and oxytocin were 73.0 ± 4.4% and 75.0 ± 3.8%, respectively. Sensitivities of the assays were 1 pg of arginine-vasopressin and 0.75 pg (0.3 μU) of oxytocin per assay tube.

The higher concentrations of arginine-vasopressin and oxytocin were confirmed in the hypothalamo-neurohypophyseal system, where these hormones are synthesized, transported and stored. Relatively high concentrations of these hormones, especially oxytocin, were detected in spinal cord. Amygdala, hippocampus, limbic forebrain and pineal body contained a certain amount of arginine-vasopressin (2-20 pg/mg protein). Oxytocin (1-7 pg/mg protein) was also detected in amygdala, pons and medulla oblongata, pineal body and midbrain. The low concentrations of these hormones were also found in cerebral cortex and cerebellum.

It is well established that the posterior pituitary hormones, vasopressin (VP) and oxytocin (OXT), are synthesized in magnocells of the hypothalamic paraventricular and supraoptic nuclei, transported in the axons to the posterior lobe of the pituitary and released there into the systemic circulation to reach the target organs. The major physiological roles of VP have been known to regulate water balance in animals (antidiuretic action) and to contract arterioles (vasopressor action), and those of OXT to contract the uterus (oxytocic action) and to stimulate milk ejection in the lactating mammary gland. Recently, these neurohypophyseal hormones have been shown to be distributed not only in the hypothalamo-hypophyseal system but widely in the central nervous system (Buijs, 1978; Buijs et al., 1978; Sofroniew and Weindl, 1978; Sofroniew and Weindl, 1978), and the suprachiasmatic nucleus was demonstrated to contain VP and its carrier protein, neurophysin (Swaab et al., 1975; Vandesande et al., 1975). The demonstration of VP in parvocellular neurons strongly suggested a neurosecretory role for this nucleus. Furthermore, there is some evidence showing that...
VP is involved in the brain function, i.e., the mechanism of memory (De Wied, 1965; De Wied, 1971), circadian rhythm in behavior (Rusak and Zucker, 1975; Stedson and Watson-Whitmyre, 1976; Zimmerman and Robinson, 1976) and regulatory mechanism of intracranial pressure (Noto et al., 1978; Noto et al., 1979) etc. However, as to OXT there is little evidence indicating its involvement in the brain function although the histochemical studies proved its occurrence in various parts of the central nervous system. Therefore, more detailed studies on the distribution of VP and OXT are needed to elucidate their physiological roles in the central nervous system.

This paper reports the determination of arginine-vasopressin (AVP) and OXT in a biological sample by radioimmunoassay using specific rabbit antisera to these hormones and data on their distribution in rat brain are presented.

Materials and Methods

Reagents

AVP (grade VI, 100 IU/ml), OXT (grade III, 200 IU/ml), bovine serum albumin (crystalized), bovine serum γ-globulin (crystalized) and bovine thyroglobulin (crystalized) were purchased from Sigma Chemical Co. Synthesized OXT (lyophilized powder) and complete Freund’s adjuvant were available from Peptide Center, Osaka and Difco Laboratories, respectively. Na\textsuperscript{125}I was supplied by New England Nuclear.

Preparation of rabbit antiserum to AVP

Nine ml of the AVP solution (100 IU/ml) was lyophilized. The dried residue and 30 mg of bovine serum albumin were dissolved in 3 ml of 0.01 M phosphate buffer, pH 7.0. One and half ml of 0.02 M glutaraldehyde was added dropwise to the mixture solution for 15 min. The reaction was promoted by gentle agitation at room temperature for 4 h and then terminated by dialysis against water at 4°C for 24 h. The dialysed solution was lyophilized. One mg of the dried residue (bovine serum albumin-AVP conjugate) was emulsified in 1 ml of complete Freund’s adjuvant and then injected intracutaneously at 40–50 points on the back of a male rabbit. Immunization was performed on each for two weeks. After 6 immunizations the blood of the animal was collected from the carotid artery and the serum was separated by centrifugation. This was kept in a frozen state at −80°C until use for radioimmunoassay.

Preparation of rabbit antiserum to OXT

Six mg of OXT (lyophilized powder) and 39.34 mg of bovine thyroglobulin were dissolved in 2.5 ml of 0.01 M phosphate buffer, pH 7.4. To the mixture solution was dropwise added 0.5 ml of 0.018 M ethylcarbodiimide for 15 min and then the reaction was promoted by gentle agitation at room temperature for 5 h. The reaction mixture was dialysed against 0.01 M phosphate buffer, pH 7.4 at 4°C for 24 h. This was lyophilized and the immunization procedure was the same as that for preparation of antiserum to AVP.

Radioiodination of AVP and OXT

To 10 μl of the AVP solution (100 IU/ml) were added 25 μl of 0.5 M phosphate buffer (pH 7.5) containing 0.5 mCi of Na\textsuperscript{125}I and then 10 μl of 0.75 % chloramine-T. The mixture solution was gently shaken at room temperature for 1 min and then 100 μl of 0.05 M phosphate buffer (pH 7.2) containing 10 % sucrose was added to it. This was applied to a 0.9×20 cm column of Sephadex G-25 (superfine) immersed previously in 0.01 M acetic acid containing 0.5 % bovine serum albumin, and developed with the acetic acid solution at 4°C. Fractions containing radioactive AVP were collected and acetic acid (5 μl to 1 ml of the eluate) was added. The acidified solution was kept in a frozen state until use for radioimmunoassay.

Radioiodination of OXT was performed using 10 μl of 1 % OXT according to the same procedure as that for AVP and radioactive moniodo-OXT was purified by gel filtration on the Sephadex G-25 column.

Extraction of AVP and OXT from brain tissue

Male Wistar rats weighing 180–200 g were sacrificed by bleeding. The brain was quickly taken out and its regions were dissected according to the brain map of König and Klippel (König and Klippel, 1967). Suprachiasmatic and supraoptic nuclei were taken from slices between A6860 μm and A5660 μm and paraventricular nucleus.
between A6280 µm and A4620 µm. Brain tissue was homogenized in an aliquot volume of 0.1 N HCl. After centrifugation of the homogenate at 3,000 rpm for 20 min, the supernatant solution was taken and to 1 ml of the solution was added 2 ml of cold acetone. This was mixed well and then centrifuged. Four ml of petroleum ether was added to the supernatant solution. After shaking vigorously, the mixture solution was centrifuged and the aqueous layer was evaporated to dryness. The dried residue was used for determination of AVP and OXT.

Radioimmunoassay of AVP and OXT

The non-equilibration method was used for the assays. The dried residue prepared from brain tissue was dissolved in 1.0 ml of 0.06 M phosphate buffer, pH 7.5, containing 1 mM disodium ethylenediaminetetraacetate, 0.2 mM cystine and 0.15 % bovine serum albumin. Two tenth ml of the solution was transferred into a coated assay tube and duplicate determinations of AVP and OXT were performed for each sample. To the transferred solution was added 50 µl of anti-AVP or anti-OXT solution which was a 20,000 times diluted rabbit antiserum with phosphate buffer, pH 7.5. The mixture solution was incubated at 4°C for 24 h and then radioiodinated AVP or OXT (50 µl, about 5,000 cpm) was added. Incubation was continued another 48 h. For separation of free from bound ¹²⁵I-AVP or ¹²⁵I-OXT, 100 µl of 1 % bovine γ-globulin and 1 ml of 20 % polyethylene glycol were added to the reaction mixture and it was centrifuged at 3,000 rpm for 30 min. The supernatant solution was discarded and radioactivity of the precipitate was counted with an automatic gamma counter (Packard Autogamma 500C).

Determination of protein

Concentrations of protein were determined according to the method of Lowry et al. (Lowry et al., 1951).

Results

Specificities and sensitivities of rabbit antisera to AVP and OXT

Fig. 1 shows a standard curve of AVP and cross-reaction curves of OXT and desglycinamide-deamino [Arg⁸]-vasopressin using the anti-AVP serum. OXT and desglycinamide-deamino [Arg⁸]-vasopressin did not react significantly with the anti-AVP serum and 1-100 pg of AVP/tube was measurable by this assay.

Fig. 2 shows a standard curve of OXT and cross-reaction curves of AVP and vasotocin using the anti-OXT serum. The cross-reaction of anti-OXT serum with AVP or vasotocin was practically negligible, and 1-200 pg (0.4-80 µU) of OXT/tube was measurable.
Fig. 2. A standard curve of OXT and cross-reaction curves of AVP and vasotocin. \( B_0 \) represents the cpm of \(^{125}\)I-OXT bound to the anti-OXT serum under no cold peptide in the assay tube. OXT, \( \bullet-\bullet \); AVP, \( \Diamond-\Diamond \); vasotocin, \( \triangle-\triangle \).

Fig. 3. Gel filtration chromatography of authentic AVP and posterior pituitary extract. Gel filtration chromatography of authentic AVP and acetone-pet.ether extract of rat posterior pituitary was performed on a 0.9×20 cm column of Sephadex G-25 (superfine) equilibrated previously with 0.06 M phosphate buffer, pH 7.5, containing 1 mM disodium ethylenediaminetetraacetate, 0.2 mM cystine and 0.15 % bovine serum albumin. One two hundredth of the posterior pituitary extract and 1.2 ng of authentic AVP was applied to the column and developed with the phosphate buffer. Fractions of 1 ml of the effluent were collected. Two tenth ml of each fraction was used to determine the amounts of AVP by radioimmunoassay.
measurable by this assay.

**Recovery rates of AVP and OXT and accuracy in the radioimmunoassays**

The recovery rate of AVP added to the acidic homogenate of the brain tissue in the radioimmunoassay was 73.0 ± 4.4% (Mean ± S.E.M.; n, 10). The within-assay and the between-assay coefficients of variation were 9.6% and 12.0% at 25 pg, respectively.

The recovery rate of OXT added to the acidic homogenate of the brain tissue in the radioimmunoassay was 75.0 ± 3.8% (Mean ± S.E.M.; n, 10). The within-assay and the between-assay coefficients of variation were 6.5% and 9.3% at 25 pg, respectively.

**Immunological identification of AVP and OXT**

In order to confirm that the assay method specifically picked up AVP or OXT in biological samples, gel filtration chromatography of the acetone-pet.ether extract of rat posterior pituitary was performed on a 0.9 x 20 cm column of Sephadex G-25 (superfine) equilibrated previously with 0.06 M phosphate buffer, pH 7.5, containing 1 mM disodium ethylenediaminetetraacetate, 0.2 mM cystine and 0.15% bovine serum albumin. The dried residue of the extract was dissolved in 10 ml of the phosphate buffer. Fifty µl of the solution was applied to the column and developed with the phosphate buffer. Fractions of 1 ml of the effluent were collected. Two tenths ml of each fraction was used to determine the amounts

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Fig. 4. Gel filtration chromatography of authentic OXT and posterior pituitary extract. Gel filtration chromatography of authentic OXT and rat posterior pituitary extract was performed by the same method as that of AVP. Fractions of 1 ml of the effluent were collected. Two tenth ml of each fraction was used to determine the amounts by radioimmunoassay.
of AVP or OXT by radioimmunoassay.

Fig. 3 shows chromatographic patterns of the extract of rat posterior pituitary and the authentic AVP. A single peak of immunoreactivity with the anti-AVP serum was observed and its elution pattern coincided well with that of the authentic AVP.

Fig. 4 shows chromatographic patterns of the extract of rat posterior pituitary and the authentic OXT. A single immunoreactive peak was observed with the anti-OXT serum and its elution pattern coincided with that of the authentic OXT.

In order to ascertain the exactness of the radioimmunoassay serial dilutions of the acetone-pet.ether extract of rat posterior pituitary and the authentic AVP or OXT sample were estimated by the assays and the dilution curves were compared with each other.

Fig. 5A and 5B show the dilution patterns of the extract and the authentic AVP or OXT sample, respectively. The dilution curves of immunoreactivities with the anti-AVP or OXT serum were proportional between the extract of rat posterior pituitary and the authentic AVP or OXT.

**Fig. 5A**

1.0

0.5

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\[ \frac{B}{B_0} \]

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\( \text{Posterior Pituitary} \)

\( 8000 \ 4000 \ 2000 \ 1000 \ 500 \ 250 \ (x) \)

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\( \bullet \) Authentic AVP

---

10

100

1000

pg/tube

**Fig. 5 B**

1.0

0.5

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\[ \frac{B}{B_0} \]

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\( \text{Posterior Pituitary} \)

\( 16000 \ 8000 \ 4000 \ 2000 \ 1000 \ 500 \ 250 \ (x) \)

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\( \bullet \) Authentic OXT

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10

100

1000

pg/tube

Fig. 5. Serial dilutions of the biological samples in the radioimmunoassays. Serial dilutions of the acetone-pet.ether extract of rat posterior pituitary and authentic AVP or OXT sample were estimated by the assays.

A. Shows the dilution patterns of the extract and authentic AVP.

B. Shows the dilution patterns of the extract and authentic OXT.
Table 1. Concentrations of arginine-vasopressin and oxytocin in various regions of rat brain.

<table>
<thead>
<tr>
<th>Region</th>
<th>Arginine-vasopressin pg/mg protein</th>
<th>Oxytocin pg/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory bulb</td>
<td>1.72±0.79</td>
<td>0.50±0.1</td>
</tr>
<tr>
<td>Limbic forebrain</td>
<td>2.33±0.95</td>
<td>2.23±0.35</td>
</tr>
<tr>
<td>Striatum</td>
<td>0.58±0.02</td>
<td>0.65±0.08</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.13±0.57</td>
<td>1.05±0.18</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>0.64±0.05</td>
<td>0.55±0.18</td>
</tr>
<tr>
<td>Amygdala</td>
<td>20.3±1.9</td>
<td>6.70±1.0</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.98±0.72</td>
<td>1.38±0.15</td>
</tr>
<tr>
<td>Midbrain</td>
<td>1.82±0.43</td>
<td>3.43±0.45</td>
</tr>
<tr>
<td>Pons and medulla oblongata</td>
<td>0.94±0.23</td>
<td>5.58±1.03</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.39±0.08</td>
<td>0.30±0.08</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>107±56</td>
<td>298±50</td>
</tr>
<tr>
<td>Supraoptic nucleus</td>
<td>1,320±50</td>
<td>237±49</td>
</tr>
<tr>
<td>Paraventricular nucleus</td>
<td>740±90</td>
<td>263±73</td>
</tr>
<tr>
<td>Suprachiasmatic nucleus</td>
<td>580±230</td>
<td>214±29</td>
</tr>
<tr>
<td>Median eminence</td>
<td>7,560±1,600</td>
<td>1,223±150</td>
</tr>
<tr>
<td>Posterior pituitary</td>
<td>52,000±8,600</td>
<td>42,250±1,000</td>
</tr>
<tr>
<td>Pineal body</td>
<td>18.9±5.5</td>
<td>4.53±0.93</td>
</tr>
</tbody>
</table>

Values represent means ± S.D. in 5 experiments.

**Distribution of AVP and OXT in rat brain**

Table 1 shows distribution of AVP and OXT in rat brain. The highest concentrations of the hormones were detected in posterior pituitary and the higher ones in median eminence, supraoptic nucleus, paraventricular nucleus and suprachiasmatic nucleus. Relatively high concentrations of the hormones, especially OXT, were observed in spinal cord. Amygdala, hippocampus, thalamus, limbic forebrain as well as pineal body contained a considerable amount of AVP while a tolerable amount of OXT was detected in amygdala, limbic forebrain, midbrain, pons and medulla oblongata and pineal body. Low concentrations of the hormones were also found in cerebral cortex and cerebellum.

**Discussion**

Since evidence for a physiological role of VP in the process of storage and retrieval of information in rat brain was provided (De Wied, 1965; De Wied, 1971), the attention of investigators, especially neurochemists, neuroendocrinologists and neuropathologists, has been focussed on the distributions of VP as well as OXT within the central nervous system. Although immunohistochemical localization studies (Buijs, 1978; Buijs et al., 1978; Sofroniew and Weindl, 1978; Sofroniew and Weindl, 1978) revealed that VP and OXT containing fibers spread out from the synthesizing nuclei in the hypothalamus to extrahypothalamic regions including the septum, amygdala and hippocampus, which are thought to be important areas to the memory mechanism, earlier attempts to measure VP in the limbic system and brain stem were unsuccessful either because of insufficient sensitivity of the radioimmunoassays or inadequate procedures used in extracting the hormone from the tissue sample. In 1978, Dogterom et al. (Dogterom et al., 1978) determined the VP and OXT concentrations of several extrahypothalamic regions in which VP and OXT containing fibers had been visualized by
immunohistochemistry, and reported that VP was detected in the hypothalamus, amygdala, septum, hippocampus, nucleus parafascicularis and medulla oblongata and OXT in the hypothalamus, septum and nucleus parafascicularis in lower amounts than VP but in higher amounts in the medulla oblongata of Wistar rats. Furthermore, recent immunohistochemical studies (Sofroniew, 1980; Buijs, 1980) on projections from VP, OXT and neurophysin neurons to neural targets demonstrated that neural target areas were found in portions of the limbic system, diencephalon, mesencephalon, brain stem and spinal cord and axosomatic as well as axodendritic contacts were detected in the neural target areas. The present study is the first systematic measurement of AVP and OXT in defined regions of the central nervous system of rats and confirmed the immunohistochemical observations about VP and OXT in the intra- and extrahypothalamic portions.

An interesting finding is that the amount of OXT is higher than AVP in the caudal parts of the central nervous system, i.e. midbrain, pons and medulla oblongata and spinal cord. The immunohistochemical study (Sofroniew, 1980) revealed that oxytocinergic fibers from paraventricular nucleus present in lamina I–III of the dorsal horn along the entire length of the spinal cord from cervical through coccygeal levels and at thoracic and upper lumbar levels pass to the sympathetic intermediolateral nucleus. Therefore, oxytocinergic fibers from the paraventricular nucleus might be involved in modification of some sensory and sympathetic functions.

Another interesting finding is the occurrence of AVP in the pineal body as well as the suprachiasmatic nucleus because the content of melatonin and serotonin and the activities of their related enzymes in the former tissue were proved to change diurnally (Quay, 1963; Klein and Weller, 1970; Axelrod, 1974) and a biological clock is thought to be located in the latter nucleus (Stedson and Watson-Whitmyer, 1976). Therefore, the vasopressinergic fibers from suprachiasmatic nucleus might project to the pineal body. The present data on the occurrence of OXT in the suprachiasmatic nucleus are contrary to those obtained in the immunohistochemical observations (Buijs, 1978; Vandesande et al., 1975). We examined OXT neurons in the suprachiasmatic nucleus immunohistochemically using our antiserum to OXT and proved that terminal-like staining was detected in the surroundings but no OXT neurons in the nucleus.

Recently, monoamine metabolism of the septum, nucleus parafascicularis and nucleus tractus solitarii, where the neuropeptides are abundant, was reported to accelerate upon intracranial applications of VP (Kovács et al., 1977; Tanaka et al., 1977; Versteeg et al., 1976). Therefore, more detailed distributions of VP and OXT in well defined regions of the central nervous system under different conditions and the effects of the neuropeptides on metabolism of the brain monoamines promise to clarify the physiological roles of VP and OXT in behavioral, neuroendocrine and autonomic processes.

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**References**


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