Local Difference in Concentration of Vasopressin and Monoamines in the Equine Median Eminence

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Synopsis

The hypophysial stalk of the horse had a typical structure of the median eminence and contained noradrenaline, dopamine, serotonin, vasopressin and paraldehyde fuchsine-stainable material. The posterior wall of the hypophysial stalk contained higher concentration of noradrenaline than the anterior and lateral walls. On the other hand, the anterior and lateral walls contained dopamine but the posterior wall did not. The concentration of serotonin was almost the same through these three portions. Vasopressin concentration was highest in the anterior wall and lowest in the posterior wall. Large droplets of paraldehyde fuchsine-stainable material were accumulated in restricted areas of the anterior and posterior walls. Fine droplets of the material were distributed in the outer layer of all the three portions of the stalk. These results indicate that there is the regional and functional differentiation in the median eminence of the horse.

The idea that the median eminence is differentiated into multiple portions with different adenohypophysiotropic functions has been proposed in mammals by Daniel and his associates (see Daniel 1966) and also in birds by Farner and his associates (see Farner et al., 1967). These investigators based their proposition mainly on the finding that each portal vessel connecting the median eminence and the adenohypophysis supplies blood to a small restricted area in the adenohypophysis. Supporting this idea, it was shown in the bird that there were differences in the concentrations of stainable neurosecretory material (Farner et al., 1967) and arginine vasotocin (Ishii et al., 1970) between the anterior median eminence and posterior median eminence.

However, such a local difference in the hormonal component in the median eminence has not been reported in the mammal. In the present study, we compared the concentration of vasopressin, noradrenaline, dopamine and serotonin between different portions of the equine median eminence. The distribution of AF-positive material in the equine median eminence was also studied.

Material and Methods

The hypophysial stalk of the horse was used as the material. This portion of the horse is equivalent to the median eminence of the other vertebrates. The tissue was dissected out from the horse within one hour after slaughtering. For a histological study, whole hypophysial stalk was fixed in the Bouin’s solution just after the dissection. For assays of vasopressin and monoamines, the tissue was kept chilled on ice and transported to the laboratory. One to two hours after the collection, the hypophysial stalk was cut into four pieces of the anterior, bilateral and posterior walls in most experiments and two lateral wall pieces were combined. In one experiment for the assay of vasopressin, the hypophysial stalk was horizontally divided into two pieces of the proximal and distal halves.

Histology

Seven hypophysial stalks were used. After the fixation, they were embedded with paraffin and
serially sectioned at 10 µ in thickness. Cross sections were prepared in five horses and sagittal sections in two horses. Sections were stained with Gomori's paraldehyde-fuchsin (AF) method.

Assay of catecholamines

The tissue was pooled from 10 to 18 animals. Each of the anterior, posterior and lateral wall was separately homogenized in a chilled 0.4 N perchloric acid solution. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 × g for 10 min. The resultant supernatant was stored in a deep freezer for one to two weeks and then used as the tissue extract for the assay.

Catecholamines were isolated from the tissue extract by the alumina adsorption method described by Anton and Sayre (1962) and then determined by the following three methods: trihydroxyindol (THI) method of Bertler et al. (1958), ethylene diamine condensation (ED) method of Weil-Malherbe (1961), and a modification of the method described by Carlsson and Waldeck (1958). Details of these isolation and determination procedures were described in our previous paper (Iwata and Ishii 1969).

Assay of serotonin

The anterior, lateral and posterior wall tissues were pooled from 8 horses. Each wall tissue was homogenized in a chilled 0.4 N perchloric acid solution containing 0.02% ascorbic acid and 0.2% EDTA-2Na. The homogenate was centrifuged in a refrigerated centrifuge at 10,000 × g for 10 min. Resultant supernatant was adjusted between pH 5 and 6 by adding a concentrated sodium carbonate solution and stored until the time of the assay. After 5 days of storage, serotonin was isolated from this extract by ion exchange chromatography using Amberlite CG 50 column. Determination of serotonin was performed by fluorometry according to the description of Andén and Magnusson (1967). In our experiment, the fluorescence was measured by Turner's fluorometer type 111. The filter combination of the fluorometer was No. 7–50 for excitation light and No. 58 for emission light.

Bioassay of vasopressin

In one experiment, regarding vasopressin concentration a comparison was made among the three portions of the anterior, lateral and posterior walls and in another experiment between the two portions of the proximal and distal halves. Each tissue was homogenized in a chilled 0.1 N hydrochloric acid solution and centrifuged in the refrigerated centrifuge. Resultant supernatant was used as the tissue extract. Vasopressin in the extract was assayed by the rat vasopressor method of Dekanski (1952). Synthetic lysine-vasopressin (Sandoz) was used as the standard sample. Assay design and calculation of potency was performed according to Holton (1948).

Results

Structure of the median eminence

The equine hypophysial stalk was in a shape of oblique circular cylinder. A wide infundibular cavity existed in the center of the stalk. The wall of the stalk consisted of four layers of the tissues arranged from the outer surface to the inner surface of the stalk in the following order: pars tuberalis cell layer, palisade layer, fiber layer and ependymal cell layer. Arrangement and constituents of these layers are basically the same as those of the other mammalian species (Kobayashi and Ishii 1969). In addition to these layers, a thin connective tissue layer intervened between the pars tuberalis cell layer and palisade layer. This connective tissue layer had four lamellar centripetal projections which divided the wall of the stalk into the anterior, bilateral and posterior portions, as shown in the cross section in Figure 1.

AF-positive material observed in the hypophysial stalk of the horse was classified into three types from the difference in shape.
and distribution. Large AF-positive droplets were densely accumulated in a certain area of the palisade layer next to the connective tissue layer (Fig. 2). This area was spread along one of the connective tissue projections and occupied such a wide space as 0.6 to 1.2 mm in length and 3 to 4 mm in width in the sagittal section. This type of accumulation was found in the posterior wall of the stalk in 5 of 7 horses, in the anterior wall in one of the remaining 2 horses and in both anterior and posterior walls in the last horse. In all cases, the area of the accumulation was restricted in the distal half of the stalk. In the palisade layer, there were a number of extremely fine droplets of AF-positive material (Fig. 3). This type (Type 2) of the material was encountered in the palisade layer of all the three portions of the stalk. The last type of the AF-positive material (Type 3) was Herring-body-like droplets scattered in the fiber layer. They are considered to be neurosecretory material contained in axons which terminate in the pars nervosa. This type of the material was the smallest in quantity among three types.

Distribution of vasopressin among portions of the hypophysial stalk

Although vasopressin was detected in all the three portions of the stalk (Table 1), there was a local difference in the concentration. The vasopressin concentration was significantly higher in the anterior and lateral walls than in the posterior wall. On the other hand, there was no significant difference in the concentration between the proximal half and the distal half of the stalk (Table 2).

Distribution of monoamines among anterior, lateral and posterior walls of the hypophysial stalk

The determination of catecholamine by ED method revealed that the catecholamine
Fig. 3. Small droplets of AF-positive material distributed in the palisade layer of the equine median eminence. (×1600)

Table 1. Concentration of vasopressin in the anterior, posterior and lateral walls of the equine hypophysial stalk

<table>
<thead>
<tr>
<th>Portion in stalk</th>
<th>Number of horses</th>
<th>Concentration of vasopressin (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Anterior wall</td>
<td>3</td>
<td>6.47</td>
</tr>
<tr>
<td>Posterior wall</td>
<td>3</td>
<td>3.50</td>
</tr>
<tr>
<td>Lateral wall</td>
<td>3</td>
<td>5.90</td>
</tr>
</tbody>
</table>

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>Df</th>
<th>Ms</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wall portion</td>
<td>14.88</td>
<td>2</td>
<td>7.440</td>
<td>27.35</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Remainder</td>
<td>1.63</td>
<td>6</td>
<td>0.272</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16.51</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Concentration of vasopressin in the distal and proximal halves of the equine hypophysial stalk

<table>
<thead>
<tr>
<th>Portion in stalk</th>
<th>Number of horses</th>
<th>Concentration of vasopressin (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Distal half</td>
<td>6</td>
<td>4.1</td>
</tr>
<tr>
<td>Proximal half</td>
<td>6</td>
<td>6.0</td>
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</table>

Analysis of Variance

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>Df</th>
<th>Ms</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portion</td>
<td>10.0</td>
<td>1</td>
<td>10.0</td>
<td>4.08</td>
<td>n.s.*</td>
</tr>
<tr>
<td>Remainder</td>
<td>24.5</td>
<td>10</td>
<td>2.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34.5</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*Not significant.

concentration was highest in the posterior wall and lowest in the anterior wall (Table 3). Since ED method is not specific enough to a certain catecholamine, we employed THI method, which is specific to noradrenaline, in the second experiment, and a method which modified Carlsson and Waldeck in the third experiment by which dopamine and noradrenaline can be estimated differentially. The second experiment revealed that noradrena-
Table 3. Concentration of catecholamine in the anterior, posterior and lateral walls of the equine hypophysial stalk revealed by the ethylene diamine condensation method. Noradrenaline was used as standard substance.

<table>
<thead>
<tr>
<th>Portion in stalk</th>
<th>Fresh weight (mg)</th>
<th>Number of animals</th>
<th>Concentration of catecholamine (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior wall</td>
<td>236</td>
<td>18</td>
<td>0.29</td>
</tr>
<tr>
<td>Posterior wall</td>
<td>215</td>
<td>18</td>
<td>0.48</td>
</tr>
<tr>
<td>Lateral wall</td>
<td>212</td>
<td>18</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The third experiment confirmed this result and further demonstrated that dopamine was concentrated in the anterior and lateral walls but not in the posterior wall, where noradrenaline concentration was highest (Table 5).

On the other hand, the distribution pattern of serotonin was different from that of either of these catecholamines mentioned above. The concentration of serotonin was much the same through the three portions of the anterior, lateral and posterior walls (Table 6).

**Discussion**

The present study demonstrated that each of vasopressin, noradrenaline, dopamine and AF-positive material showed more or less localized distribution in certain portions of the hypophysial stalk of the horse. These results help Daniel (1966) and Farner et al. (1967) to consider that the median eminence differs in its portions in the adenohypophysiotropic function. Recently, McCann and his co-workers (Schneider and McCann 1969; Kamberi et al., 1970) have demonstrated that among catecholamines only dopamine accelerated the release of releasing factors of luteinizing hormone and follicle stimulating hormone from the median eminence in vitro. They concluded that dopamine acted on the neurosecretory terminals in the median eminence and regulated the secretion rate of these releasing factors. The present result with dopamine suggests that such dopaminergic mechanism is localized in the anterior and lateral walls of the hypophysial stalk in the horse. The noradrenergic nerve, although its role in the median eminence is not clear at the present time, is considered to be localized mainly in the posterior wall.

In contrast to dopamine and noradrenaline, serotonin was evenly distributed throughout...
the stalk. It is possible that the serotoninergic nerve is related to the regulation of the general metabolism of all the adenohypophysial cells or a certain parenchymal cell type which is uniformly distributed in the adenohypophysis. In addition, it is noteworthy that the concentration of serotonin in the median eminence of the horse is higher than that (0.45μg/g tissue) in the equine hypothalamic tissue without the median eminence (Iwata and Ishii, unpublished data) and that (0.7μg/g tissue) in the rat hypothalamus (Fuxe 1965). This result suggests that serotonin may play some important role in the regulation of the adenohypophysial activity at the level of the median eminence.

We recognized three different types of AF-positive material in the hypophysial stalk of the horse. Type 2 is similar in shape to the AF-positive material in the anterior median eminence of the bird and type 1 to the AF-positive large droplets in the posterior median eminence of adenohypophysectomized pigeon (Kobayashi et al., 1966). Furthermore, the distribution of types 2 and 1 in the equine median eminence coincides approximately with that of the corresponding material in the avian median eminence. Further study is required to elucidate the physiological role of these AF-positive materials.

Acknowledgments

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Reference