ABUNDANCE AND LOCATION OF $^{125}$I-SALMON CALCITONIN BINDING SITE IN RAT BRAIN

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Very recently, Nakanishi et al. (1) predicted the complete amino acid sequence of pituitary pro-opiocortin in terms of the genetic code. According to the prediction, the precursor protein would contain amino acid sequences of ACTH, MSHs and β-endorphin in the C-terminal half of the molecule. The N-terminal half would be mainly composed of sequences for which biological functions are unknown. While comparing the sequences of the latter half with those of the known calcitonins from various animal species, we noticed that a common skeletal structure, basic to that of the calcitonins occurs in the sequence corresponding to -104 to -73 of pro-opiocortin; namely, cysteine at Positions 104 and 98 with a proline at Position 73. Thus, we assumed that in the tissue where a fragment of the C-terminal half exists, a calcitonin-like peptide may also occur and display some physiological function. In fact, the co-production of ACTH and calcitonin by peptide-producing tumors has been observed (2, 3). We selected the brain and examined whether or not there is any specific binding site for calcitonin. For the binding assay, salmon calcitonin-I was employed instead of rat calcitonin, because the ultimobranchial calcitonin is biologically more active and stable than mammalian calcitonins (4, 5).

$^{125}$I-Salmon calcitonin-I was prepared by the method of Marchalonis (6). The specific activity was adjusted to be about 0.10 Ci/μmole. Whole brain or various regions from male Wistar rats (150–170 g) were homogenized in 50 mM Tris-HCl buffer (pH 7.4). After standing at 4°C for 30 min, the homogenate was centrifuged at 15,000 × g for 10 min. The pellet was once washed with the buffer and finally suspended in the buffer for binding assay. Typically, an assay mixture in 1.0 ml of 50 mM Tris-HCl buffer (pH 7.4) contained pellet suspension (1.6 mg as protein), 20 nmole of bacitracin, 1 pmole of $^{125}$I-calcitonin with or without 1 nmole of cold calcitonin. After incubation at 37°C for 5 min, the tube was centrifuged and the radioactivity in pellet was estimated. Due to strong binding of $^{125}$I-calcitonin to the glass fiber disc, the centrifugation method was used for this assay. Here the specific binding means cold-calcitonin-replacable binding. As shown in Table 1, rat brain tissue universally contained a specific binding site for calcitonin. The pH optimum for the binding was around 7.4. The content of the site appears to be the highest in hypo-

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thalamus and the lowest in cerebellum, and cortex, but even the latter region had more binding sites than the kidney, a target tissue for the peripheral hormone action of calcitonin (7). Scatchard plot analysis of whole brain homogenate (Fig. 1) shows that in the homogenate of whole brain, there are at least two types of binding sites (K_D and Bmax for low affinity site are 10 nM and 440 fmole/mg protein and those for high affinity site 0.57 nM and 56 fmole/mg protein, respectively).

The results obtained here, though of preliminary nature, provided the first biochemical indication of the presence of calcitonin binding site in the central nervous system. It is highly possible that the site mediates some of behavioral effects of centrally given calcitonins, such as analgesia (8-12), tremor (12) and decreased eating (13). Further studies are expected

### TABLE 1. Regional distribution of salmon calcitonin binding in the rat brain

<table>
<thead>
<tr>
<th>Tissues</th>
<th>¹₂⁵I-calcitonin bound (cpm x 10^3/mg protein) absence or presence of 1 µM calcitonin</th>
<th>specific binding (fmole/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole brain</td>
<td>6.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Kidney</td>
<td>5.4</td>
<td>4.4</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>8.7</td>
<td>3.6</td>
</tr>
<tr>
<td>Striatum</td>
<td>5.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Midbrain</td>
<td>6.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>5.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Medulla Oblongata</td>
<td>5.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Pons</td>
<td>5.4</td>
<td>3.6</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>4.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Cortex</td>
<td>5.3</td>
<td>4.0</td>
</tr>
</tbody>
</table>

a) Each value is the mean of two determinations, each being estimated by quadruplicate assay on tissues from two rats.
b) Each value is the mean of two determinations, each being estimated by quadruplicate assay on pooled brain areas from four rats.

![Scatchard plot of specific binding of ¹₂⁵I-calcitonin as a function of its concentration. The concentration of ¹₂⁵I-calcitonin in the assay mixture was changed from 0.25 nM to 4.0 nM. Each point is the mean of two determinations, each being estimated by triplicate assay on brain tissues from two rats.](image)
to define for which function of the brain the binding site exists and whether or not there is any brain-born calcitonin.

REFERENCES


