GROWTH HORMONE-RELEASING ACTIVITY IN THE HYPOTHALAMI OF KITTENS WITH LESIONS OF THE REGION OF THE PRAVENTRICULAR NUCLEI

By
S. Sawano, A. Arimura, A. V. Schally,

ABSTRACT

An attempt was made to evaluate the growth hormone (GH)-releasing activity in extracts of the stalk-median eminence region (SME) from kittens with bilateral lesions of the paraventricular nuclei, which showed a marked growth retardation, and from sham-operated animals. SME extract from the sham-operated control kittens induced a significant decrease in pituitary GH content. However, SME extract from kittens with bilateral lesions in the region of the paraventricular nuclei failed to induce the depletion of pituitary GH content. The cerebral cortical extracts from both groups elicited no effect on pituitary GH. These results show that the hypothalamus of the lesioned kittens has no GH-releasing activity, i.e. GH-releasing factor (GRF) is lacking. This suggests that, in kittens, the area of the paraventricular nuclei plays an important role in the synthesis of GRF, so that destruction of this area causes growth impairment.

Experimental growth disturbance can be induced in animals by damage to the hypothalamus (Bogdanove & Lipner 1952; Endroczi et al. 1956; Reichlin 1960, 1961; Hinton & Stevenson 1962; O’Brien et al. 1962, 1964; Bach et al. 1964). It has been thought that hypothalamic lesions may interfere with the secretion of growth hormone (GH) by the pituitary gland. Reichlin (1961) showed that,

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in rats with extensive hypothalamic lesions, pituitary GH content decreased significantly. O'Brien et al. (1962, 1964) and Bach et al. (1964) reported that destruction of the region of the paraventricular nucleus induced a marked growth impairment in kittens. Recent observations have clearly demonstrated the existence of GH-releasing factor (GRF) in the hypothalamus of animals (Deuben & Meites 1965; Ishida et al. 1965; Krulich et al. 1965; Pecile et al. 1965; Schally et al. 1965, 1966, 1967 a, b; Machlin et al. 1967; Sawano et al. 1968) and humans (Muller & Pecile 1966; Schally et al. 1967 a, b). However, little is known of the relationship between growth impairment and GH-releasing activity in the hypothalamus of animals with hypothalamic lesions. The purpose of the present study was to investigate the GH-releasing activity of the hypothalamus in kittens subjected to bilateral lesions of the paraventricular nuclei.

**MATERIALS AND METHODS**

**Preparation of kitten stalk-median eminence (SME) extract**

Eight kittens weighing from 500 to 700 g were divided into 2 groups of 4 each. In one group electrolytic lesions were made bilaterally in the region of the paraventricular nuclei. Stereotaxic coordinates were corrected according to the skull size of the kittens. Another group was sham-operated as the control. All kittens used in this study were force-fed a complete liquid diet on a strict basis of a fixed volume of nutrient per gram of body weight; no voluntary access to other food or water was permitted. Ten to fourteen days after the surgery, the kittens were killed with ip injections of pentobarbital (Nembutal®). The fragment of tissue containing mainly the stalk and the median eminence of the tuber cinereum (SME) was carefully dissected. The area removed was located between the posterior margin of the optic chiasma and the premammillary nuclei and extended 0.5–1.0 mm toward the brain base. The dissected SME or cerebral cortex (CC) of the same group of animals were pooled and homogenized in ice-cold 0.1 N HCl. The homogenates were centrifuged at 3000 rpm for 20 min at 4° C and the supernatants decanted into other test tubes. The pH of the supernatant was adjusted to 7.4 by adding 1 N NaHCO₃ solution. The final volume was made up with 0.9% saline so that 1.0 ml of the extract contained one SME (average weight 25 mg) or a comparable amount of CC.

**Test for GH-releasing activity of the extract**

Charles River CD® female rats (130–140 g body weight), which were obtained from Charles River Breeding, Brookline, Mass., were used as the recipient animals of the test samples. The extent of depletion of pituitary GH content after the injection of the samples was used as the index of GH-releasing activity in the extracts (Pecile et al. 1965). They were given 0.9 ml saline, SME extract or CC extract by intracarotid injection under ether anaesthesia. Fifteen minutes after the injection they were decapitated. The anterior pituitary glands of each group were pooled and homogenized in 0.9% saline. After being appropriately diluted, the pituitary homogenates were kept frozen until assayed for GH activity.
GH assay
GH activity of the samples was measured by the "tibia test" method of Greenspan et al. (1949). Sprague-Dawley female rats, hypophysectomized 26 days after birth, were obtained from Hormone Assay Labs., Chicago, Ill. Six to eight were used to assay each sample. NIH-GH-S7 was used as the reference standard. The GH potencies of the pituitary homogenates were calculated by a 4-point assay according to Finney (1952). Significance of the differences between the groups was determined by factorial analysis (Finney 1952).

Histological preparations
After removal of the SME and a portion of CC, the brains were fixed in 10% formol saline. After fixation, frozen sections of the midbrain were prepared and stained with cresyl violet. The anterior pituitary glands were fixed in 10% formol saline, and the sections prepared with stained azocarmine and aniline blue.

RESULTS

GH-releasing activity in lesioned kittens
As shown in Table 1, the intracarotid injection of SME extract from the sham-operated control kittens (Group 2) induced a significant decrease in

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment of recipient rats</th>
<th>AP equivalents/ assay rat total dose/ 4 days (mg)</th>
<th>Growth hormone evaluation</th>
<th>( P ) vs saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Width of tibia cartilage* ( \mu \pm S. E.** )</td>
<td>Potency GH/mg pit.</td>
<td>95% Fiducial limits</td>
</tr>
<tr>
<td>1.</td>
<td>Saline</td>
<td>189 ± 2.6</td>
<td>50.1</td>
<td>39.2–60.5</td>
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<td></td>
<td></td>
<td>231 ± 2.6</td>
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<tr>
<td>2.</td>
<td>0.9 SME from sham-operated kittens</td>
<td>171 ± 2.4</td>
<td>37.2</td>
<td>27.1–46.9</td>
</tr>
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<td></td>
<td></td>
<td>212 ± 2.4</td>
<td></td>
<td>0.001</td>
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<tr>
<td>3.</td>
<td>0.9 SME from lesioned kittens</td>
<td>191 ± 3.5</td>
<td>49.2</td>
<td>39.2–59.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>230 ± 2.1</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>4.</td>
<td>Cerebral cortex from sham-operated kittens</td>
<td>228 ± 3.0</td>
<td>46.0</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td>234 ± 3.8</td>
<td></td>
<td>NS</td>
</tr>
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</table>

\* 6–8 hypophysectomized assay rats per group.
\** Mean value of tibia width of hypophysectomized rats was 148 ± 3.1.
pituitary GH content in recipient rats as compared with that of saline-injected animals (Group 1) \((P < 0.001)\). This indicates the presence of GRF in the hypothalami of the sham-operated kittens. However, the injection of SME extract from the kittens with hypothalamic lesions (Group 3) failed to deplete the pituitary GH content in the recipient rats. Similarly, the CC extracts (Groups 4 and 5) had no effect on the pituitary GH content. These results suggest that SME extracts from the kittens with bilateral paraventricular lesions have no GRF.

**Histological findings**

In general the location and size of the hypothalamic lesions were comparable to those reported previously \((O'Brien et al. 1962, 1964; Bach et al. 1964)\). More than half of the region of the paraventricular nucleus was destroyed bilaterally in all but one of the four kittens; the paraventricular nucleus was intact on one side in the latter kitten. Other structures partially involved included the medioventral nucleus of the thalamus, the parvocellular nucleus, the dorso-median nucleus, the anterior periventricular nucleus, and the dorsal hypothalamic area. The ventral part of the hypothalamus was intact in all four kittens. A typical lesion is shown in Fig. 1.

Examination of serial sections of the pituitary glands from kittens with lesions and from controls failed to reveal any significant difference in number and granulation of acidophils.

**D I S C U S S I O N**

\(O'Brien et al. (1962, 1964)\) and \(Bach et al. (1964)\) reported earlier that kittens with induced bilateral destructive lesions in the region of the paraventricular nucleus showed a marked reduction of growth rate within the first two post-operative weeks, despite the fact that these animals were provided with exactly the same amount of food per gram of body weight as the controls. They also noticed that administration of GH restored the impaired growth rate to levels similar to those of sham-operated controls. On the other hand, destruction of any part of hypothalamus, except in the region of the ventromedial nucleus, resulted either in hypophagia or aphagia. The most extreme aphagia was associated with the destruction of the lateral hypothalamic areas, in which instances the kittens were force-fed for two or three weeks before signs of spontaneous feeding behaviour began to appear. Those kittens in which the region of the ventromedial nucleus was destroyed usually became aggressive and, despite their restricted, force-fed liquid diet, tended to store significantly greater amounts of fat than did the controls. They also observed that severe pituitary acidophilic degranulation occurred in the pituitary glands of the
This photomicrograph was taken about half-way through serial sections from the brain of a kitten in the »PV lesion« group. It corresponds approximately to frontal plane 12.4 mm in Bleier's atlas of the cat diencephalon (Bleier 1961). The cut edge in the medial basal portion represents the border of the »SME« removed at the time of sacrifice.

kittens with lesions of the paraventricular area despite intact hypophysial circulation and normal PBI. The absence of pituitary degranulation in the present study may be explained by the short post-operative period. The kittens reported previously (Bach et al. 1964) were sacrificed ten to fourteen months following lesioning. Perhaps changes in granulation of acidophils require more time to develop.

The present study revealed that the SME extract of sham-operated kittens contained GH-releasing activity like normal kittens (Muller et al. 1967), while SME from the kittens with bilateral paraventricular lesions lacked significant GH-releasing activity. These results suggest that growth retardation in kittens, induced by destruction of the region around the paraventricular nuclei, may result from impaired synthesis of GRF in the hypothalamus. This also suggests that the paraventricular area plays an important role in the synthesis of GRF.

The question arises as to whether or not the SME is also damaged at the
time of placement of the paraventricular lesions. SME, dissected to test the GH-releasing activity in the present study, contained the area from the posterior margin of the optic chiasma to the premammillary nuclei and extended 0.5–1.0 mm in depth toward the brain base. The paraventricular nuclei were not included in the preparation of the SME. As shown in the histological preparation (Fig. 1), the lesions were essentially limited to the paraventricular area. In addition, it has been shown in previous studies (O'Brien et al. 1962, 1964; Bach et al. 1964) that the hypophysial vasculature was intact and thyroid function was normal. This may indicate that at least synthesis of thyrotropin-releasing factor is not affected. However, a contribution of thyrotropin deficiency to the observed growth failure cannot be ruled out completely.

It can be argued that non-specific stress induced by placement of lesions in the hypothalamus may affect growth. The kittens with an equivalent lesion elsewhere in the hypothalamus showed the same growth rate as compared with the sham-operated controls (Bach et al. 1964). Therefore, it is possible to exclude the effects of non-specific stress on growth in these kittens.

Meites & Fiel (1965) have studied the effect of starvation on hypothalamic GRF content. They reported that starvation markedly decreased the capacity of the rat hypothalamus to evoke pituitary GH release. Therefore, it had to be considered whether temporary appetite loss in the lesioned kittens, induced by the surgical procedure, affected the GH-releasing activity in the hypothalamus. In the present experiments, the food intake of sham-operated kittens was comparable to that of lesioned animals. On the tenth to fourteenth day, when these kittens were sacrificed, the lesioned kittens already seemed to recover from the surgical stress and their appetites were restored to the pre-operative levels. In addition, SME from sham-operated kittens, which showed similar patterns of appetite to the lesioned kittens after the operation, had significant GRF activity. These findings indicate that the lack of GRF in the hypothalamus of the lesioned kittens is not due to malnutrition, but, to the paraventricular lesions.

The lesions reported by Endroczí et al. (1956), which affect growth in rats, were in the ventral hypothalamus and involved the supraoptic, preoptic, and paraventricular nuclei up to the anterior margin of the tuber. Reichlin (1960, 1961) reported that a marked growth disturbance in rats was induced only by extremely large lesions involving both anterior and middle hypothalamus; however, destruction limited to the area between paraventricular nucleus and optic chiasma did not necessarily prevent normal growth when the animals were given thyroxine. Hinton & Stevenson (1962) stated that, in rats, ablation of the hypothalamus in the region bounded by the supraoptic nucleus and dorsal aspect of the optic tract (and perhaps extending as far medially as the suprachiasmatic nuclei and anteriorly to the preoptic nucleus) produced a retardation of growth, which was accompanied by thyroid hypofunction. On
the other hand, replacement therapy with thyroxine, when compared with that of controls, failed to increase the growth rate of the kittens with lesions of the paraventricular areas (O'Brien et al. 1964; Bach et al. 1964). The existing evidence suggests that lesions limited to the paraventricular areas, histologically confirmed, interfere with growth because of a reduction in GRF production. Lesions involving more anterior hypothalamic areas may retard growth because of interference with thyrotrophin secretion.

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REFERENCES


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