Extrahypophysial distribution of corticotropin as a function of brain size

(pituitary/hypothalamus/immunoreactive corticotropin/hypophysectomized rats)

ROBERTA MOLDOW AND ROSALYN S. YALOW

Veterans Administration Hospital, Bronx, New York 10468; and The Mount Sinai School of Medicine, City University of New York, New York, New York 10029

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ABSTRACT

Determination by radioimmunoassay of corticotropin in the brains of rats, rabbits, dogs, monkeys, and human beings reveals that the dimensions within which the hormone is found is about the same for each of these species but that the anatomical regions in which the hormone is found depends on brain size. Corticotropin is widely distributed in the brain of rats but is found only in the hypothalamic region of the primate brain. The patterns of immunoreactivity observed after Sephadex gel filtration confirm that the molecular forms of corticotropin found in extrahypophysial regions are similar to those in the pituitary of each species. These findings suggest that the mammalian pituitary is the sole site of synthesis of the hormone. The observation of persistence of corticotropin in the brains of commercially hypophysectomized rats has been interpreted by others as suggesting diencephalic as well as pituitary origin for this peptide. However, our studies demonstrate that 8 weeks after hypophysectomy the rats we have received from commercial sources manifest stress-stimulated plasma corticotropin concentrations about 80% of that found in intact rats in spite of the fact that residual pituitary tissue was not found by visual inspection of the sella. Scrapings from the sella revealed a corticotropin content up to 5% that of the average rat pituitary.

It is now experimentally demonstrable that there are a group of peptides, such as somatostatin (1, 2), substance P (3), vasopressive intestinal peptide (4), and cholecystokinin or its COOH-terminal octapeptide (5, 6), which are found in the gastrointestinal tract and in the central nervous system. Evidence has also been accumulating that peptide hormones, such as β-lipotropin, corticotropin (ACTH), and peptides structurally related to them, initially thought to be of pituitary origin, are found widely distributed in the brain in extrahypothalamic regions (7–10). The suggestion that these peptides are in fact synthesized in other than the pituitary gland is based on the observation that such activity persists in the brain of rats for some time after hypophysectomy (8–11). In this report we describe the distribution of immunoreactive ACTH in the brains of rats, rabbits, dogs, monkeys, and human beings and demonstrate that the dimensions within which the ACTH is found is about the same for each of these species but that the anatomical regions appears to depend on brain size. ACTH is widely distributed in the brain of rats but is found only in the hypothalamic region of primate brain.

METHODS

Brains were removed from adult Sprague-Dawley rats, New Zealand white rabbits, dogs, and rhesus monkeys immediately after they were killed. A single human brain was obtained 6 hr after death. To evaluate the effect of the time after death on apparent ACTH content, samples from monkey brains were obtained during a craniotomy procedure, immediately after they were killed and also 6 hr after death. In all cases, the pituitary gland and pituitary stalk were identified and removed separately. Generally, regions of the brain including the cerebellum, cerebral cortex (frontal, parietal, and occipital), striatum, hippocampus, amygdala, medulla, pons, midbrain, preoptic area, thalamus, and hypothalamus were identified, dissected, and immediately frozen and stored at −50 °C until used. In some instances brains were immediately frozen on dry ice and dissected while still frozen. Samples were weighed and minced while still frozen, and ACTH was extracted in 10 volumes of boiling water as described (12, 13). To determine the form of immunoreactive ACTH in the tissue extracts, we fractionated aliquots on Sephadex G-50 superfine columns by methods previously described (12–14). Columns were equilibrated and eluted with 0.1 M NaCl containing 0.3% 2-mercaptoethanol and 0.25% human serum albumin at 4 °C. The nature of the immunoreactivity eluting in the void volume was compared with that of material with the same apparent elution volume (big ACTH) obtained by fractionation of lung tumor extracts (13). Immunoreactive ACTH was absorbed from the extracts with 5 mg of Quso G32 and eluted from the Quso with 40% acetic acid in 0.1 M HCl. This procedure extracts authentic 1–39 peptide (little ACTH) from plasma or other fluids. The eluates were dried in a gentle stream of nitrogen at 45 °C and then were redissolved in the standard diluent used for assay. Sephadex gel filtration was used to determine the hormonal forms in the original extracts, in the residual after Quso extraction, and in the material eluted from the Quso.

Radioimmunoassay of ACTH was performed by methods in general use in our laboratory (12–15). Purified human ACTH, synthetic human ACTH (supplied by the National Pituitary Agency, and porcine ACTH (Schwarz/Mann, 135 units/mg) can be used interchangeably for labeling and as standards since they appear to have identical immunoreactivity with our antisera (guinea pig 330-5-27).

Twenty-four hypophysectomized Sprague-Dawley rats were obtained from a commercial source (Charles River, Cambridge, MA). One week after hypophysectomy, blood was sampled by cardiac puncture after ether stress on all the rats. Eight were then killed and the completeness of the hypophysectomy was evaluated by visual inspection of the sella. The same procedure was repeated 1 week later and 8 of the remaining 14 rats were then killed. Blood was again sampled after ether stress 8 weeks after hypophysectomy on the last five rats, and the animals were then killed. Although visual inspection suggested that the hypophysectomy was complete in all these animals, scrapings of the sella confirmed the presence of ACTH-containing frag.

Abbreviation: ACTH, corticotropin.
RESULTS

The regional distribution of immunoreactive ACTH in the brains of rats, rabbits, dogs, monkeys, and human beings is shown in Table 1. In all species studied, ACTH was found in the hypothalamus; its concentration in the ventral region was always greater than in the dorsal region. Whether or not ACTH is detected in a particular region of the brain appears to depend on its physical distance from the hypothalamo-pituitary area (Fig. 1). Thus, ACTH was found in the hypothalamus, midbrain, pons, medulla, thalamus, preoptic area, and limbic system of rats and rabbits; in the hypothalamus, preoptic area, midbrain, amygdala, and thalamus of dogs; but only in the hypothalamus of monkeys and human beings (Table 1). Therefore it would appear that the wider regional distribution in the rat and rabbit brains is a consequence of the smaller size of the brain in these species. Furthermore, the location of the pituitary gland in the rat is relatively posterior so that it is actually ventral to the midbrain as well as to the hypothalamus (Fig. 1).

The concentrations and hormonal form of ACTH in the brain tissue appeared to be quite stable. Thus, there were no significant differences in the ACTH contents of dog or rabbit brains when the tissues were dissected from a fresh brain at room temperature or when the brain was quick frozen and then dissected while frozen (Table 1). The ACTH content of monkey dorsal hypothalamus did not differ significantly whether the tissue was obtained during a craniotomy procedure, immediately after the animal was killed, or at autopsy (Table 1). The higher ACTH content in the ventral hypothalamus obtained

<table>
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<tr>
<th>Brain region</th>
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* Dissected frozen.
* ND = not detectable, <1 ng/g.

FIG. 1. Distribution of concentrations of immunoreactive ACTH in the pituitary and brain of several animal species. The rat, dog, and human brains are drawn to scale. The regions shown in circles have been enlarged to show in better detail the concentrations of ACTH in the brain of the rat and dog. ACTH was not detectable in the regions shown in white. Abbreviations: Pit, pituitary gland; VH, ventral hypothalamus; DH, dorsal hypothalamus; POA, preoptic area; MB, midbrain; M, medulla; Th, thalamus; Hippo, hippocampus.
from the monkey immediately after it was killed is attributed to a more limited ventral dissection of this area. Immunoreactivity was not detectable in the extrahypothalamic areas of the primate brains (<1 ng/g of wet weight tissue, Table 1) even when samples were obtained at craniotomy or immediately after sacrifice.

The different molecular forms of ACTH found in the extrahypophysial regions were similar to the forms found in the pituitary for each species (Fig. 2). It has previously been shown (16) that an additional hormonal form with an elution volume on Sephadex G-50 midway between the void volume and the elution volume of the 1–39 peptide is found in the pituitary of rabbits and rats, mammals whose primary glucocorticoid is corticosterone. The brain extracts of these species contain all three hormonal forms (Fig. 2). Intermediate ACTH is not prominent in the pituitaries or in the brain extracts of dogs, monkeys, and humans, species whose primary glucocorticoid is cortisol (Fig. 2).

Generally, less than 20% of total immunoreactivity was present in the void volume eluates in those tissue extracts in which ACTH was readily measurable. However, Sephadex gel filtration of extracts in which ACTH was barely measurable or undetectable revealed only void volume immunoreactivity. Since nonspecific factors can interfere in the immune system, we questioned whether this void volume immunoreactivity was authentic big ACTH.

The nature of the void volume immunoreactivity found in brain extracts in which ACTH was barely detectable was compared with that of authentic big ACTH obtained from lung tumor extracts. The major fraction of immunoreactive ACTH is absorbed from extracts of normal rat hypothalamus, dog thalamus, and lung tumor by Quso and is eluted from Quso by acetone/HCl (Fig. 3). The monkey cortex, which contained no measurable ACTH (Table 1), appeared to have a void volume component of immunoreactivity after Sephadex gel filtration (Fig. 3). However, this immunoreactive peak was not adsorbable by Quso as was authentic big ACTH. A minor fraction of void volume immunoreactivity from the dog thalamus also behaved inappropriately on adsorption to and elution from Quso. Thus, some brain extracts contain what appears to be void volume immunoreactivity but this is more likely to be due to nonspecific interference in the antigen–antibody reaction rather than to the presence of authentic big ACTH. Quso adsorption as herein described or tryptic conversion, a technically more difficult procedure (12, 13), should be used to assess the possibility of such artifacts.

The presence of pituitary hormones in the brains of commercially prepared hypophysectomized rats has been taken as evidence for de novo synthesis of pituitary hormones in the brain (8–11). We have observed that in these animals residual pituitary tissue is rarely detected upon visual inspection of the sella. Nonetheless, although there is an immediate decrease in stress-stimulated ACTH release in hypophysectomized rats, within 2 months the plasma ACTH concentrations in such animals after stress stimulation are about 80% of the level found in intact rats (Fig. 4). It would appear therefore that visual inspection of the sella is not sufficient to ensure that the hypophysectomy has been total. Scrapings from this region had an ACTH content ranging from about 1 to 5% of the normal pituitary content. This is of particular importance since the hypothalamic ACTH content is only about 0.1% that of the pituitary. Thus, there is good reason to believe that the pituitary gland is still the source of ACTH in the brains of these presumably "hypophysectomized" rats.

**DISCUSSION**

The data on the distribution of immunoreactive ACTH in the brains of large and small mammals presented in this report are consistent with the hypothesis that the pituitary is the sole site of synthesis of this peptide. The regions of the brain in which the ACTH is found are more extensive in rats and rabbits than...
in the species with larger brains, but there is no reason to assume that the synthetic mechanism is different in these small-brained animals. It would appear that the distance from the pituitary at which ACTH is found and the concentration gradient are roughly comparable in all species but that the particular regions of the brain included are species-dependent. The hypothesis that the pituitary is the source of the ACTH-like peptide found elsewhere in the brain is strengthened by the observations that the relative distribution of the various molecular forms found elsewhere in the brain resembles the distribution found in the pituitary.

Our experience with commercially hypophysectomized rats and with those we have prepared is that visual inspection of the sella is insufficient to assure absence of all pituitary fragments. It is recommended that other methods of testing be used to evaluate the completeness of pituitary removal. Ether-stress testing seen after hypophysectomy is not as revealing as is testing at a later period. Scraping of the sella and immunoadsorbent of its ACTH content are also useful procedures. In intact rats the hypothalamic content is only 0.1% that of the pituitary, so that small remaining pituitary fragments can be the source of ACTH even in presumably totally hypophysectomized animals.

If our hypothesis that ACTH is synthesized only in the pituitary is valid, then some consideration must be given as to how it reaches various regions of the brain. To explain this phenomenon of transport of ACTH to the brain, we are completely dependent on the observations of others. There are several conceivable routes by which pituitary ACTH can reach the brain: directly by retrograde flow along the portal vessels to the median eminence or by leakage in the basal cistern or indirectly via the systemic circulation. We consider that indirect transport via the systemic system is unlikely since the brain ACTH content of a rat bearing an F3 pituitary tumor and having a 100-fold increase in plasma ACTH was no greater than that of a control rat (unpublished studies).

It had previously been considered that blood flow in the portal vessels was solely from the hypothalamus to the pituitary (17). However, recent observations (18, 19) are consistent with earlier evidence (20) that had suggested retrograde flow. ACTH in human and monkey brains appears to be restricted solely to the hypothalamic region and retrograde flow alone could account for its presence. Other regions of the brain are more distant and protected from leakage by the position of the pituitary, which is well seated in a bony structure. The rat pituitary is ventral both to the hypothalamic and midbrain region and, from its gross anatomy, is less isolated from the brain. In the rat, as in the larger species, retrograde flow may occur. In addition there is a possibility for leakage into the cerebrospinal fluid in the basal cistern (21). Thus, pituitary hormones could reach other regions such as preoptic area, midbrain, pons, and medulla in addition to the hypothalamus.

These findings taken together suggest that the mammalian pituitary is the sole site of synthesis of ACTH. Similar studies are required to determine if it is also the sole site for synthesis of other pituitary hormones or their fragments.
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