

## Corticotrophin-like Bioactivity in the Pituitary Gland and Brain of the Pacific Hagfish, *Eptatretus stouti*

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The cytochemical bioassay for corticotrophin (ACTH) was used in an attempt to detect ACTH-like activity in the Pacific hagfish, *Eptatretus stouti*. Extracts of the pituitary gland and brain were active in this assay system but those of liver and skeletal muscles were not. The slopes of the dose-response lines of the pituitary extracts were less than those of the mammalian corticotrophin standard preparation but greater than those of the brain extracts. The results suggest that the corticotrophic factor in the hagfish differs from mammalian ACTH. © 1985 Academic Press, Inc.

Various adrenocortical steroids, including corticosterone and cortisol, have been detected in blood serum from the Atlantic hagfish, *Myxine glutinosa* and the Pacific hagfish, *Eptatretus stouti* (Chester-Jones and Phillips, 1960; Phillips *et al.*, 1962; Weisbart *et al.*, 1980). Their concentrations are low in comparison with other vertebrates and their tissue source is uncertain (Weisbart and Idler, 1970; Weisbart *et al.*, 1980). Nevertheless corticosteroid secretion may be under the influence of the pituitary gland, as it is in more advanced species, for the serum concentration of corticosterone is raised by repeated injections of mammalian corticotrophin (ACTH) (Idler *et al.*, 1971; Weisbart *et al.*, 1980). However, attempts to demonstrate ACTH in the brain and pituitary gland of this species by immunocytochemical methods have not been successful, possibly because it differs structurally from its mammalian counterpart and is hence not recognized by antisera raised against porcine ACTH (Nozaki and Gorbman, 1983). Accordingly, we have used a sensitive biological assay method in an attempt to detect ACTH-like

activity in extracts of various tissues from *E. stouti*.

### MATERIALS AND METHODS

**Animals.** Pacific hagfish, *E. stouti*, were trapped at Bamfield, Vancouver Island, Canada, in January 1982 and again in February and May 1983. Sexually differentiated adults of both sexes with body weights ranging from 100 to 150 g and from 30 to 45 cm in length were used mostly on the day of capture (228 hagfish). Another fifty hagfish were transported to Seattle where they were kept unfed in covered plastic tanks in aerated seawater at 8-10°C for 3 months. The hagfish were anesthetized by immersion in 0.4% MS 222 dissolved in seawater. Pituitary glands were collected from all 278 animals and brains from 50 of them; samples of liver, body wall muscle (m. parietalis), and the cephalic portion of the same muscle mass were collected from 20 animals in each sample. The samples were immediately frozen on dry ice and lyophilized.

Lyophilized tissue was homogenized in 1.0 ml 0.1 M HCl and stored at 4°C for 24 hr to achieve maximum extraction of ACTH activity (Hodges and Vernikos, 1960). After centrifugation (1875g for 5 min) the supernatant fluid was decanted, neutralized with 0.1 M NaOH and used immediately.

**Cytochemical bioassay of corticotrophin.** ACTH activity was assessed by the sensitive cytochemical method (Alaghband-Zadeh *et al.*, 1974) which depends upon the ability of the pituitary hormone to evoke oxidation of ascorbic acid in the zona reticularis of

guinea pig adrenal glands and thus to alter the reducing potential of the tissue. Third Corticotrophin International Working Standard (porcine 1-39 ACTH), of which 10  $\mu\text{g}$  is equivalent to one International Unit, was used as the standard preparation. The mean ( $n = 10$ ) index of precision (standard deviation of the slope/slope =  $\lambda$ ) =  $0.12 \pm 0.03$ .

*Statistics.* Indices of precision were calculated and tests for parallelism were performed as described in *British Pharmacopoeia* (1980).

## RESULTS

Corticotrophin (1-39 ACTH) altered the reducing potential of the zona reticularis in sections of guinea pig adrenal tissue and, over a range of concentrations from 5 to 500 nanounits/ml, there was an inverse linear relationship between the logarithm of the concentration of the peptide and the response. Extracts of the pituitary gland and brain from *E. stouti* diluted 1 in  $10^2$ –1 in  $10^5$  were also active in this respect; but those of liver, body wall muscle, and head muscle were not. The slopes of the dose–response of the pituitary extracts (1.82,  $\lambda = 0.17$  and 1.94,  $\lambda = 0.19$ ) were significantly ( $P < 0.01$ ) less than those of the standard preparation (2.45,  $\lambda = 0.12$ ) but greater than those of brain extracts (1.39,  $\lambda = 0.31$ ) (Fig. 1). Three other batches of pituitary extracts, prepared and tested at a different time, behaved similarly (data not shown).

## DISCUSSION

Although the cytochemical bioassay for the determination of corticotrophin is well established (Alaghband-Zadeh *et al.*, 1974; Buckingham and Hodges, 1974, 1977a and b; Holdaway *et al.*, 1974; Daly *et al.*, 1979; Gillham *et al.*, 1981; Buckingham, 1982), it has been exploited only recently in studies on ACTH and ACTH-related peptides in lower vertebrates (Baker and Buckingham, 1983). The method has many advantages, for it is not only accurate and highly precise, but also considerably more sensitive than other biological or radioimmunological techniques. Furthermore, in this bioassay system, as in others based on adrenal ascorbic acid depletion (Storring *et al.*, 1984),

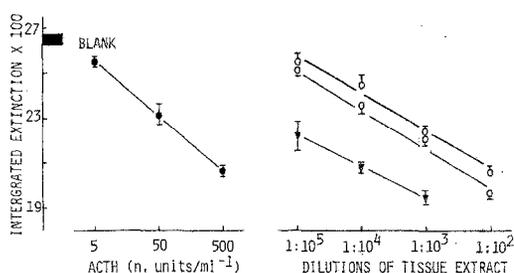


FIG. 1. Changes in reducing potential (expressed as extinction  $\times 100$ ) in the zona reticularis of sections of guinea pig tissue in response to the addition to the incubation medium of corticotrophin (porcine 1-39 ACTH, ●), pituitary extracts (○) and brain extracts (▲). Each point is the mean of 10 determinations and is shown with its standard error.

the activities of various ACTH-related molecules can be distinguished on the basis of their dose–response lines (Baker and Buckingham, 1983). Such distinction cannot be achieved with assays based on steroidogenesis where, surprisingly, the dose–response lines of ACTH-related molecules are always parallel with the standard preparation (Sayers *et al.*, 1975; Rance and Baker, 1981).

The present data demonstrate for the first time the presence of corticotrophic-like activity in the pituitary gland and brain of the Pacific hagfish. The activity appears to be specific to those tissues and was not found in the several other regions of the body tested. The marked difference in the slopes of the dose–response lines of the pituitary and brain extracts and those of the standard preparation may reflect the presence of a combination of ACTH and related peptides of the melanocyte-stimulating hormone (MSH) type (Buckingham and Baker, 1983). Alternatively, the active substance in the hypophysis may be structurally distinct from mammalian 1-39 ACTH while the activity in the brain extracts may be attributable to a substance distinct from that in the pituitary gland. This would explain the apparent absence of immunoreactive ACTH and ACTH-related peptides in the adenohypophysis of this species (Nozaki and Gorbman, 1983), for antisera raised

against porcine ACTH would be unlikely to recognize that of the fish.

The deviation from parallelism of the dose-response lines of the extracts and porcine ACTH prevents accurate quantification of the tissue content of the hormone. Nevertheless, the corticotrophic activity of the hagfish hypophysial extracts appears to be considerably less than that of mammals where higher dilutions of extracts of single glands (1 in  $10^6$ –1 in  $10^8$ ) are necessary to produce graded, submaximal responses in this assay system (Buckingham and Hodges, 1977a). This difference does not necessarily indicate that the pituitary gland is unimportant in the regulation of adrenocortical function in the Pacific hagfish. Considerable amounts of ACTH-like activity may have been lost in the extraction process; and in any event, the tissue content of hormone does not always reflect its secretory capacity (Hodges and Jones, 1964; Buckingham and Hodges, 1974). Furthermore, the ACTH-receptors in the guinea pig adrenal cortex may be unresponsive to hagfish corticotrophin. However, those on the adrenocortical cells of the Pacific hagfish, unlike those in guinea pig, may be relatively insensitive to porcine 1-39 ACTH (Idler *et al.*, 1971; Weisbart *et al.*, 1980) but highly sensitive to their native hormone. Clearly further studies are required to test these possibilities and hence to determine the role, if any, of the pituitary gland in the regulation of adrenocortical function in *E. stouti*.

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