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Two distinct corticotrophin releasing activities of vasopressin

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1 The effect of various corticotrophin releasing factors (CRFs) on the secretion of corticotrophin (ACTH) by segments of rat anterior pituitary tissue has been studied *in vitro*.

2 ACTH release was stimulated by CRF-41 (5.0×10^{-9}–2.0×10^{-7} M), hypothalamic extracts (0.2–1.6 HE ml^{-1}) and arginine vasopressin (AVP, 5.0×10^{-9}–8.0×10^{-7} M). The slopes of the dose-response lines of CRF-41 were greater than those of AVP, less than those of hypothalamic extracts from control animals and resembled those of hypothalamic extracts from Brattleboro rats.

3 Simultaneous addition of AVP (10^{-10} M) to the incubation medium enhanced the response to CRF-41 and increased the slope of its dose-response lines. The adrenocorticotrophic response to CRF-41 was enhanced similarly by pretreatment of the tissue with AVP (1.25×10^{-11}–2.0×10^{-10} M) as also was the response to hypothalamic extracts. In contrast, pretreatment of the tissue with CRF-41 (2.3×10^{-11}–2.3×10^{-8} M) depressed the subsequent response to CRF-41 and to hypothalamic extracts.

4 The marked difference between the concentrations of vasopressin required to facilitate maximally the response to CRF-41 and those necessary to evoke ACTH release suggests that the two effects may be mediated by different types of vasopressin receptor.

Introduction

Corticotrophin releasing factor (CRF) appears to comprise several components which act synergistically (Gillies *et al.*, 1982). These include a 41-residue peptide (CRF-41), arginine vasopressin (AVP), adrenaline and an unidentified fraction(s) (Vale *et al.*, 1981). The importance of vasopressin has been emphasised repeatedly (Gillies & Lowry, 1979; Buckingham & Leach, 1980; Buckingham 1981; Gillies *et al.* 1982; Rivier & Vale, 1983; Berkenbosch *et al.*, 1984) but its mode of action is still controversial. It may act directly on the corticotrophs to stimulate corticotrophin (ACTH) release (Gillies & Lowry, 1979) or it may facilitate the activity of other, more potent, corticotrophin releasing factors (Buckingham, 1981). In the present work these possibilities have been investigated further by examining the effects of CRF-41 and AVP, alone and in combination, on the secretion of ACTH by adenohypophysial segments *in vitro*.

Methods

Animals

Male Sprague-Dawley rats (SPF, bred at the Royal Free Hospital School of Medicine from a colony derived from Charles River) and homozygous Brattleboro and Long Evans (control) rats (Charing Cross Hospital Medical School) were used. Animals, weighing between 75 and 100 g, were housed 5 per cage for at least 7 days before the start of each experiment in a room with controlled lighting (lights on 07 h 00 min–19 h 00 min) in which the temperature was maintained at 21–23 °C. The animals were handled regularly and food and water were available *ad libitum*. They were always killed between 07 h 30 min and 10 h 30 min in order to avoid any changes associated with the circadian rhythm.

Determination of corticotrophin releasing activity

Adenohypophyses, removed from decapitated Sprague-Dawley rats, were divided into 4 segments of approximately equal size and incubated for 2 h 30 min at 37 °C in 2.0 ml of an artificial medium ionically similar to cerebrospinal fluid (CSF) (Bradbury *et al.*, 1974) containing 10^{-3} M ascorbic acid, pH 7.4, through which 95% O_2/5% CO_2 was continually passed. The medium was replaced after 2 h and 2 h 15 min. The segments were then separated and incubated for a further 15 min in either medium (1.0 ml) containing the test substance (hypothalamic extracts, CRF-41 or arginine vasopressin) or a corresponding volume of medium alone (controls). The medium was stored at −70 °C and its ACTH content determined subsequently. In some experiments, two segments from each gland were transferred to medium containing AVP or CRF-41 during the second incubation period (2 h–2 h 15 min.). The other two (controls) were incubated in medium alone.

Hypothalamic extracts

Hypothalami were removed from Sprague-Dawley rats immediately after decapitation. Each gland was homogenized in 20 µl 0.1 M HCl and stored on ice for 1 h; 0.2 ml artificial CSF (Bradbury *et al.*, 1974) containing 10^{-3} M ascorbic acid (Gillies *et al.*, 1982) was then added, the mixture shaken thoroughly and centrifuged at 1.875 *g* for 5 min. The supernatant fluid, which was always used within 1 h, was stored at 4 °C and diluted immediately before use. In some experiments (Figure 1) hypothalamic extracts were also prepared from homozygous Brattleboro rats and appropriate (Long Evans) controls.

Peptides

Ovine CRF-41 (Dr Vale and Dr Rivier, Salk Institute, California) and arginine vasopressin (Cambridge Research Biochemicals) were dissolved and diluted in artificial CSF (Bradbury *et al.*, 1974) containing 10^{-3} M ascorbic acid (Gillies *et al.*, 1982) immediately before use. The solutions did not exhibit any intrinsic activity in the cytochemical assay or influence the responses to ACTH.

Determination of corticotrophin

ACTH was estimated by the sensitive, specific and precise (index of precision = 0.092 ± 0.021 for 10 dose response lines, fiducial limits (P = 0.95) = 93.5–107.0 (n = 5)) cytochemical bioasssay method (Alaghband-Zadeh, *et al.*, 1974) using IIIrd Corticotrophin I.W.S. as the standard preparation.

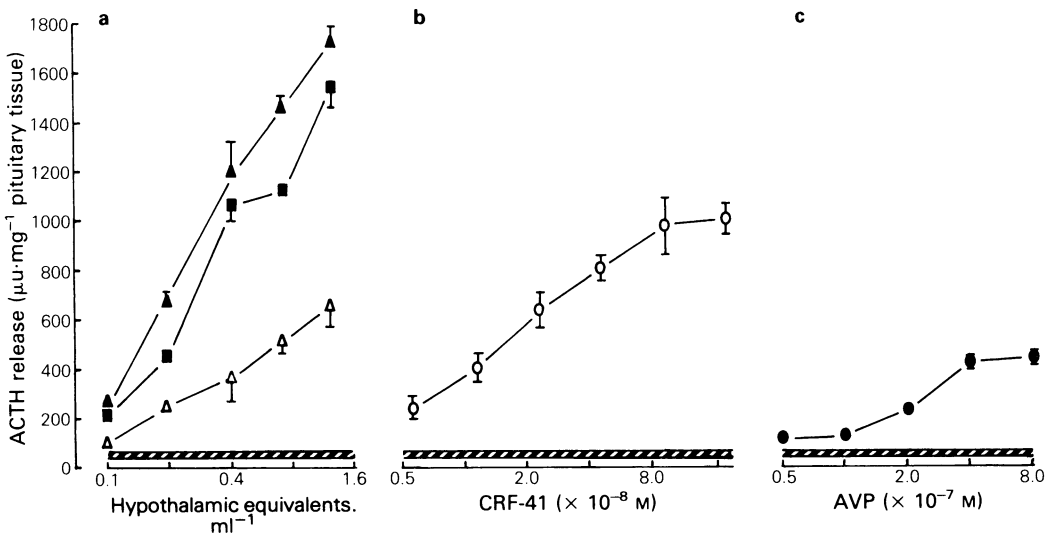

Figure 1 The effect of graded doses of (a) hypothalamic extracts prepared from Sprague-Dawley (▲), Long Evans (■) or homozygous Brattleboro rats (△), (b) CRF-41 (○) or (c) arginine vasopressin (AVP, ●) on the release of ACTH from segments of anterior pituitary tissue *in vitro*. ▨ = ACTH release from untreated anterior pituitary segments. Each point is the mean of 6 determinations and vertical lines show s.e. Standard errors omitted were within ± 15% of the mean.

Statistics

Results were analysed using analysis of variance, Duncan's multiple range test and the test for parallelism recommended in the British Pharmacopoeia (1980).

Results

Hypothalamic extracts (0.1–1.6 hypothalamic equivalents. ml^{-1}) from normal (Sprague-Dawley and Long Evans) and Brattleboro rats, CRF-41 (5.0×10^{-9}–2.0×10^{-7} M) and AVP (5.0×10^{-9}–8.0×10^{-7} M) stimulated the release of corticotrophin from segments of pituitary tissue preincubated in artificial CSF (i.e. not exposed previously *in vitro* to vasopressin). Their effects were dose-related. The slopes of the dose-response lines of CRF-41 were significantly ($P<0.001$) greater than those of AVP, less than those of both control extracts (which were identical) and like those of Brattleboro extracts. These results are shown in Figure 1.

Addition of vasopressin to the final incubation medium, in a concentration (10^{-10} M) which was considerably lower than that required to evoke ACTH secretion directly, potentiated significantly ($P<0.01$, Duncan's test) the adrenocorticotrophic response to CRF-41. It also increased significantly ($P<0.01$) the slope of the dose-response lines of CRF-41, although the maximal effects of the two peptides together were still less than those of normal (Sprague-Dawley) extracts (Figure 2).

The response to CRF-41 was facilitated similarly ($P<0.01$, Duncan's test) by pretreatment of the segments with AVP (10^{-10} M). Pretreatment with vasopressin (10^{-10} M) also enhanced the subsequent adrenocorticotrophic response to hypothalamic ex-

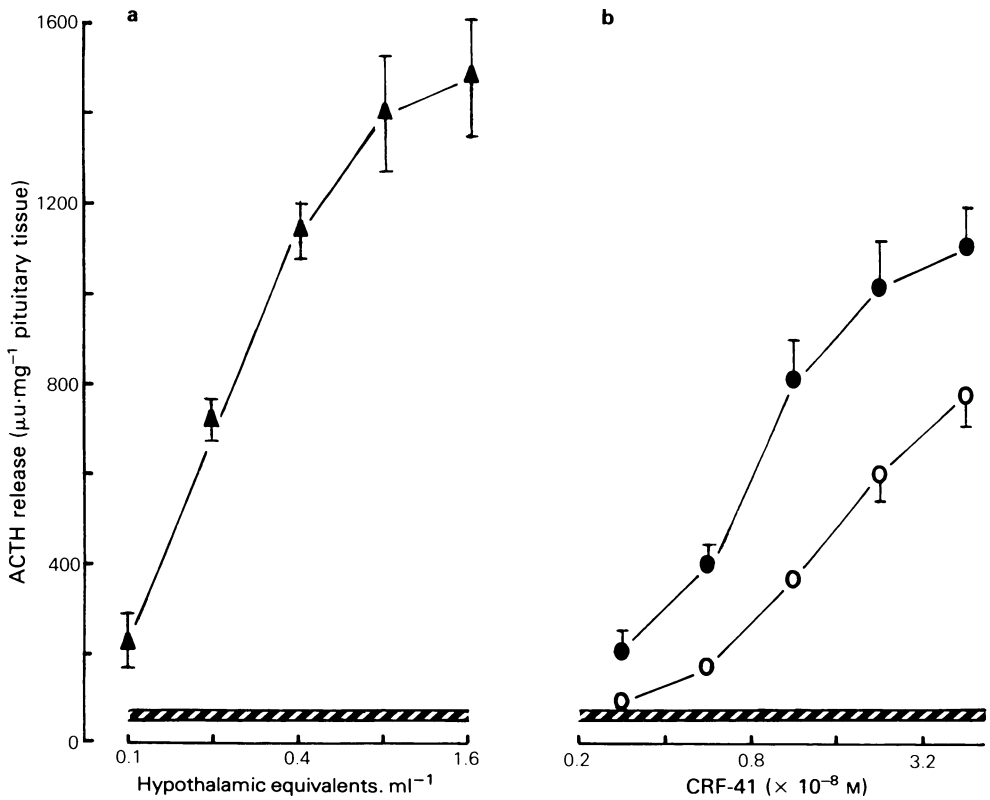

Figure 2 The effect of graded doses of (a) hypothalamic extracts prepared from Sprague-Dawley rats (▲), (b) CRF-41 (O) or CRF-41 + 10^{-10} M arginine vasopressin (●), on the release of ACTH from segments of anterior pituitary tissue *in vitro*. ▨ = ACTH release from untreated pituitary segments. Each point is the mean of 6 determinations and vertical lines show s.e. Standard errors omitted were within ± 15% of the mean.

tracts but did not alter significantly ($P > 0.2$) the slope of the dose-response lines (Figure 3).

Over a range of concentrations from 1.25×10^{-11}–10^{-10} M, the effects of vasopressin on the adrenocorticotrophic response to the subsequent addition of CRF-41 (10^{-8} M) or hypothalamic extracts (0.2 hypothalamic equivalents · ml^{-1}) to the incubation medium were dose-related. They were maximal at concentrations well below those required to stimulate ACTH release directly. In contrast, pretreatment of the adenohypophysial segments with CRF-41 (2.3×10^{-11}–2.3×10^{-8} M) depressed significantly ($P < 0.01$, Duncan's test), in a dose-related manner, the subsequent pituitary responses to CRF-41 and hypothalamic extracts. Vasopressin-induced ACTH production was not influenced by pretreatment of the tissue with either AVP (1.25×10^{-11}–2.0×10^{-10} M) or CRF-41 (2.3×10^{-11}–2.3×10^{-8} M). These results are shown in Figure 4.

Although pretreatment of the pituitary tissue with a maximal sensitizing concentration of vasopressin (10^{-10} M) enhanced the amount of ACTH released in response to the subsequent addition to the incubation medium of CRF-41 (10^{-8} M), it did not affect significantly ($P > 0.2$, Duncan's test) the adrenocorticotrophic response to CRF-41 (10^{-8} M) and AVP (10^{-10} M) given together (Figure 5).

Discussion

The results illustrate the ability of CRF-41 to stimulate the secretion of biologically active ACTH by adenohypophysial segments *in vitro*. The activity of CRF-41 differs markedly from that of hypothalamic extracts from normal animals. Indeed, its dose-response relationships are similar to those of hypothalamic extracts from Brattleboro rats and its activity, like that of Brattleboro extracts (Buckingham, 1981), is enhanced by vasopressin which supports the suggestion (Buckingham & Leach, 1980; Buckingham, 1981) that AVP is essential for the full expression of hypothalamo-pituitary-adrenocorticotrophic activity. Similar observations have been made using different *in vitro* systems although methods employing tissue segments (Turkelson *et al.*, 1982; Knepel *et al.*, 1984) are less sensitive to the 41-residue peptide than those using cultured (Vale *et al.*, 1981) or acutely dispersed (Gillies *et al.*,

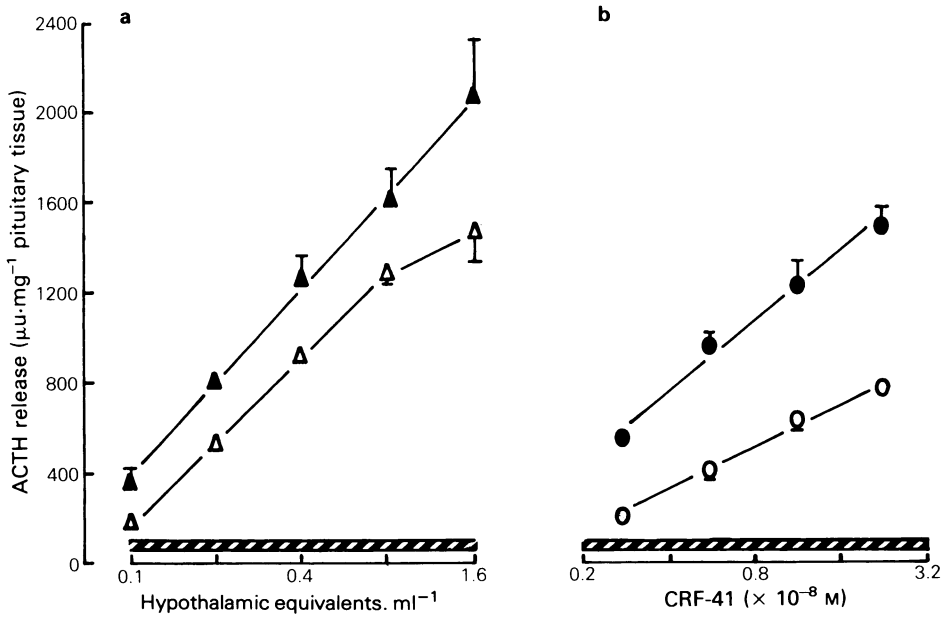

Figure 3 The effects of pretreatment of pituitary segments with arginine vasopressin (AVP, 10^{-10} M) on the subsequent adrenocorticotrophic responses of pituitary segments to graded doses of (a) hypothalamic extracts prepared from Sprague-Dawley rats or (b) CRF-41. Closed symbols, tissue pretreated with AVP; open symbols, controls; triangles, hypothalamic extracts; circles, CRF-41; hatched bar, ACTH released from untreated pituitary segments. Each point is the mean of 6 determinations and vertical lines show s.e. Standard errors omitted were within ± 15% of the mean.

1982) cells, probably because of differences in accessibility to the receptors. The data, in contrast to those of Gillies *et al.* (1982), also indicate that pretreatment of the pituitary tissue with CRF-41 reduces its subsequent responsiveness not only to the neuropeptide itself but also to hypothalamic extracts. The desensitization may be explained by down-grading of CRF-41 receptors as was suggested by Reisine & Hoffman (1983) but other possibilities, such as prolonged association of the ovine peptide with rat receptors, should not be ignored.

The activity of vasopressin is quite different from

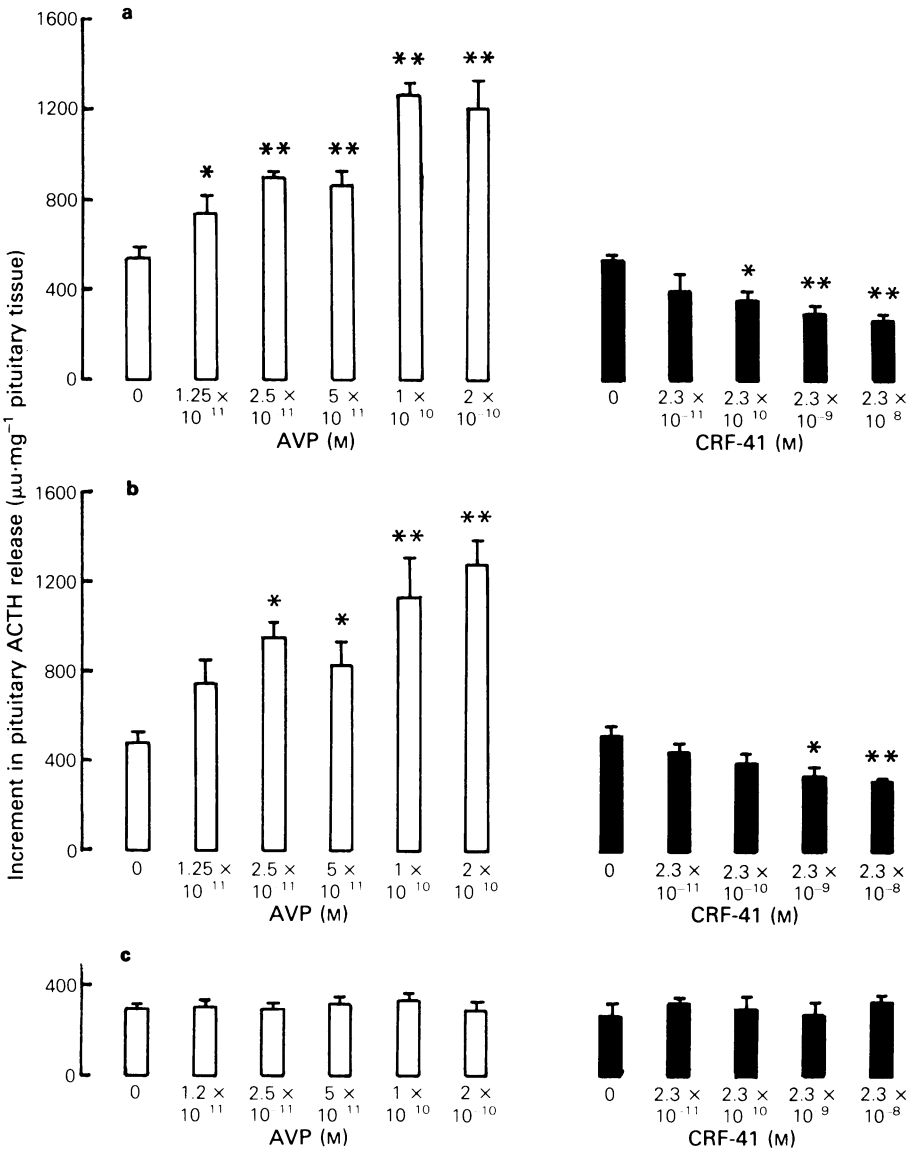

Figure 4 The effects of pretreatment of pituitary segments with graded doses of arginine vasopressin (AVP, open columns) or CRF-41 (closed columns) on the subsequent adrenocorticotrophic response to submaximal doses of (a) CRF-41 (10^{-8} M), (b) hypothalamic extracts prepared from Sprague-Dawley rats (0.2 hypothalamic equivalents. ml^{-1}) and (c) AVP (10^{-7} M). Each point is the mean of 8 determinations and is shown with its standard error. $^{*}P < 0.05$, $^{**}P < 0.01$, significantly different from control tissue (Duncan's test).

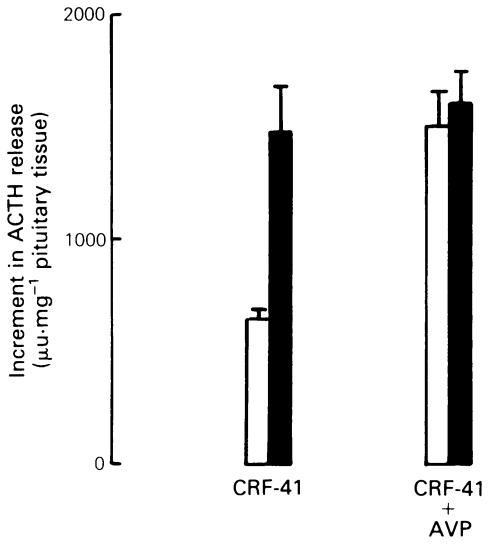

Figure 5 Effects of pretreatment of pituitary segments with a maximal 'sensitizing' dose of arginine vasopressin (AVP; 10^{-10}M) on the subsequent adrenocorticotrophic response to CRF-41 (10^{-8}M) and CRF-41 (10^{-8}M) + AVP (10^{-10}M). Closed columns, tissue pretreated with AVP; open columns, controls. Each value is the mean of 10 determinations and is shown with its standard error.

that of CRF-41. In very high concentrations it stimulates the release of ACTH in this and many other systems both *in vivo* (de Wied, 1961) and *in vitro* (Buchingham & Hodges, 1977; Gillies & Lowry, 1979; Aizawa *et al.*, 1982; Rivier & Vale, 1983). However, the present data show that vasopressin, in minute concentrations, also facilitates the adrenocorticotrophic response of the tissue to CRF-41 and to hypothalamic extracts. This is apparent when the drugs are applied together or when the tissue is pretreated with vasopressin. The ability of AVP to potentiate the subsequent adrenocorticotrophic activity of pituitary segments (Buckingham & Hodges, 1977) may reflect its retention, in small amounts, by the tissue (Lutz-Bucher *et al.*, 1977). Certainly, the responses to CRF-41 and AVP given together are not enhanced by pre-incubation with a maximal sensitizing dose of vasopressin although those to hypothalamic extracts, which contain large amounts of CRF-41 and AVP, are. Despite the remarkable interaction between CRF-41 and vasopressin, the two together still exhibit less adrenocorticotrophic activity than hypothalamic extracts from normal animals emphasizing further the multifactorial nature of the releasing factor complex.

The enormous difference between the concentrations of vasopressin required to stimulate ACTH release and those necessary to facilitate maximally the corticotrophic response to CRF-41 suggests that the two effects are mediated by different mechanisms and possibly by different types of receptor. It is generally agreed that there are at least three types of vasopressin receptor in the body (Schwartz & Livingstone, 1964; Berde & Boissonnas, 1968; Cort *et al.*, 1977; 1981). Attempts to classify those in the pituitary gland have not yet been successful (Mormède, 1983; Baertschi *et al.*, 1984; Knepel *et al.*, 1984) probably because of a failure to differentiate between the two quite distinct actions of vasopressin on ACTH secretion. The *in vitro* system described in this paper provides an ideal model for studying the separate effects of vasopressin on adrenocorticotrophic activity and is now being exploited, using selective agonists and antagonists, to classify the relevant receptors. Once this has been achieved it will be possible to study hypothalamo-pituitary -adrenocortical activity in rats which have been treated with appropriate specific antagonists and, hence, to elucidate the true physiological role of vasopressin in the regulation of ACTH secretion.

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