Adrenocorticotrophic hormone and cyclic adenosine monophosphate effect on mouse adrenal cortical cell membrane potential

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Summary. Adrenocorticotrophic hormone (ACTH) and cyclic adenosine monophosphate (cAMP) both caused a rapid and transient depolarization of the resting membrane potential of superfused rat adrenal cortical cells. The membrane depolarization to both secretagogues were very similar. The membrane potential changes occurred as early as 0.1 min and were dose dependent in both onset and extent of depolarization.

Alterations in cell membrane permeability to ions has been suggested as a mechanism by which hormones interact with target cells. In the case of adrenal cortical cells there is evidence that variations in intracellular ion concentration can alter adrenal corticosteroid production[2-5]. In vitro manipulation of extracellular ionic composition can also have a profound influence on basal and stimulated adrenal hormone secretion[6-10]. There is now some evidence that there is an actual movement of ions across adrenal cell membranes when the adrenal cells are stimulated to secrete hormone[11]. A number of important criteria however, have yet to be met in order to establish a causal relationship between membrane permeability to ions and adrenal steroid secretion. It must be shown, at least in some cells, that membrane permeability alterations occur prior to steroid secretion. Electrophysiological techniques are well suited to the study of rapid changes in ion movement across cell membranes. Previous electrophysiological studies have failed to demonstrate a significant, rapid change in membrane potential of adrenal cortical cells under normal in vitro conditions[12-13].

Adult mouse adrenal cortical tissue was superfused with a Krebs-Henseleit-glucose solution (maintained at 37 °C) at a rate of 1 ml/min. All other experimental conditions were similar to those described by Matthews and Saffran[13]. Utilizing 3M KCl-filled glass microelectrodes (resistance range of 30–100 MΩ) the resting membrane potential (RMP) was -78.2 ± 1.3 (283 cells). Membrane potentials from single adrenal cells could be monitored for up to 8 h with the techniques employed.

The addition of adrenocorticotrophic hormone (ACTH; Acthar, Armour and Co.) 0.01 to 10.0 milliunits/ml (total of 10 ml at 1 ml/min) consistently resulted in rapid and transient depolarization of the RMP (e.g. figure 1). There were significant depolarizations at all doses of ACTH employed. The mean MP increment of depolarization ranged from 2.8 mV for the lowest dose (0.01 milliunits/ml) to 8.0 mV for the highest dose (10.0 milliunits/ml) employed. The larger the dose of ACTH the more rapid the onset of depolarization. With the larger doses, the onset of depolarization was apparently instantaneous in many cells. The time delay from the 1st appearance of ACTH in the superfusion bath to an observable and consistent increment in MP ranged from 0 min to over 10 min with most of the doses used. This later observation indicates that there may be a variation in the sensitivity of individual adrenal cells to the same dose of secretagogue. Thus, a recruitment of secretory cells may partially account for the dynamics of the steroidogenic response to stimulation. The very rapid onset of depolarization in the majority of cells is consistent with the criterion of an alteration in membrane permeability prior to the onset of detectable steroid secretory activity.

Was this change in membrane potential observed with ACTH the direct result of the secretagogue interaction with the cell membrane or might the phenomena be an indirect result, possibly as the consequence of cyclic adenosine monophosphate (cAMP) interaction with the cell membrane? cAMP, or its dibutryl derivative, elicited responses similar to those observed for ACTH. The addition of cAMP (10 ml of 10 µM to 1 mM solutions) to the superfusion system resulted in a significant depolarization at all doses (figure 2). With the lowest dose there was a mean increment of 3.6 mV, whereas with the highest dose a mean MP change of 20.3 mV was observed. Again, similar to the observations made when ACTH was employed, the onset of depolarization was dose-dependent. There was a mean delay of 5.95 min for the lowest dose and 0.10 min for the highest dose. The range of delay of onset of depolarization was 0 min (more evident with the higher doses) to 20 min (in a few cells when 10 µM secretagogue was employed). The vast majority of cells tested exhibited MP changes earlier than 1 min after the first amounts of cAMP reached the tissue bath.

The overall patterns of response to cAMP and ACTH were similar. ACTH, however, appeared to be a more potent

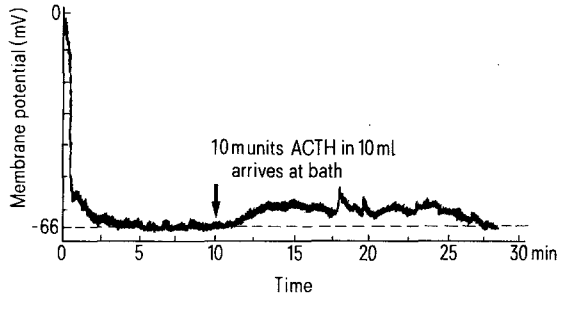

Fig. 1. Intracellular recording of a superfused mouse adrenal cortical zona fasiculata cell with a resting membrane potential of −66 mV. The addition of 1 milliunit/ml (10 ml total) ACTH results in relatively rapid and transient depolarization.

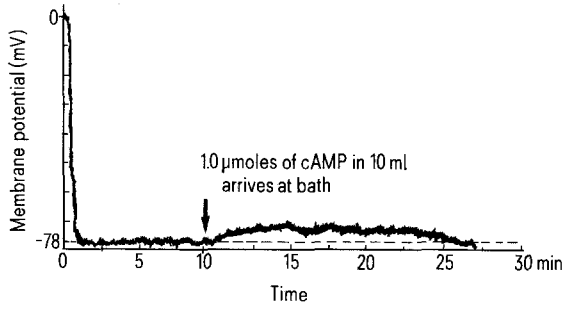

Fig. 2. Tracing of intracellular recording of a superfused mouse adrenal cortical zona fasiculata cell possessing a resting membrane potential of −78 mV. The addition of 0.1 µmole/ml (10 ml total) cAMP results in a very rapid and relatively transient membrane depolarization.

agent. ACTH was effective at approximately 0.25 nM concentration, whereas the minimum effective dose of cAMP was 10 μM. The steroidogenic response of rodent adrenal cortical tissue in our superfusion system seemed to parallel the relative membrane potential response for the respective secretagogue.

These data clearly demonstrate that both ACTH and cAMP can alter a parameter indicative of membrane permeability of individual adrenal cortical cells prior to synthesis and secretion of corticosteroids. These findings support the suggestion that a primary, rate-limiting step in the stimulus-secretion coupling of an external agent on these steroid secreting cells is one which involves the flux of ions across the cell membrane. The fact that both ACTH and cAMP can cause the membrane permeability alteration indicates that a mechanism similar to that invoked for neural transmitter action on post-synaptic membranes[14-16] may be postulated for steroid secreting cells; the peptide hormone interacts with the adrenal cell membrane causing a net increase in cAMP production. The cyclic nucleotide then interacts with the adrenal cell membrane to alter membrane permeability to specific ions resulting in an influx of, e.g. Ca^{++} ions into the cell. This ion influx can then have direct action on the intracellular biochemical machinery. The relative insensitivity of externally applied cAMP as compared to ACTH in causing both steroidogenic and membrane potential response suggests that the cyclic nucleotide acts at a membrane site from within the cell and is thus subject to phosphodiesterase activity. Alternatively, ACTH at low concentrations may have a direct action on the adrenal cell membrane permeability independent of elevations of cyclic nucleotide.

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Comparison of intradermal pigeon crop-sac bioassay and double antibody radioimmunoassay for rat prolactin[1]

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Summary. On an absolute basis, the intradermal pigeon crop-sac bioassay (PCA) gave results that were 20.5% higher than the radioimmunoassay (RIA) in rat anterior pituitary (AP) preparations. A highly significant correlation (r = 0.87) was obtained between RIA (in μg) and PCA (in Reece-Turner units) when 58 medium samples obtained by culturing rat APs in vitro were assayed for prolactin (PRL) content.

The local pigeon crop-sac assay (PCA) developed by Reece and Turner[4] resulted in good agreement with RIA when purified rat PRL was used[5,6]. However, when unpurified material was assayed, PCA always gave higher values than those obtained with RIA[5]. Available reports also indicate that the correspondence between the 2 assay systems varies according to the secretory state of the gland when anterior-pituitary (AP) extracts are used[7,8]. On the other hand, comparison of the biological and immunological activites of rat prolactin (PRL) that was secreted in vitro gave highly significant correlations between both assays[8,9]. In the present study, an attempt was made to compare RIA and PCA values on an absolute basis using crude rat AP preparations and also to establish a relationship for the 2 assay values on a relative basis using medium samples containing secreted PRL by AP explants in vitro.

Prolactin levels in crude anterior pituitary (AP) homogenates as estimated by radioimmunoassay (RIA) and pigeon crop-sac intradermal bioassay (PCA)

AP homogenate	RIA* (μg)	PCA* (μg)	RIA estimate PCA estimate
1	175.50	239.86	0.73
2	157.50	159.00	0.99
3	385.50	553.96	0.70
4	491.00	646.95	0.76

* Correlation coefficient (r) for the 2 assay values is 0.89.

Materials and methods. The PRL activity in crude rat AP preparations and in medium samples was assayed in common pigeons by the intradermal method[10]. In making comparisons on an RIA-PCA absolute basis, medium with known RIA-PRL potency was used as a standard and injected over the right crop-sac of 4 pigeons and over the left crop-sac of 4 additional pigeons in each assay. Crude AP preparations were injected over the left crop-sac of the first 4 pigeons and over the right-crop-sac of the 4 additional pigeons. The total amount of PRL in the APs in μg was calculated by estimating the crop-sac response of the medium sample and for the AP homogenate. This enabled us to compare the PRL contents in the AP preparations estimated by RIA and PCA on an absolute basis.

Comparison between the 2 assay values on a relative basis was made by estimating the PRL activity in 58 medium samples obtained by culturing AP explants in vitro by RIA (in μg units) and by PCA (Reece-Turner units).

Results and discussion. Comparison on an absolute basis. In 4 AP preparations tested, PCA gave results that were approximately 20% higher than those of RIA (table). The