Isolated Adrenal Cells as a Tool for Investigating the Mode of Action of Adrenocorticotrophin Analogues

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Isolated rat adrenal cells (Sayers et al., 1971; Richardson & Schulster, 1972; Lowry et al., 1973) offer an ideal opportunity for studying the steroidogenic response in a system that preserves cellular integrity while allowing direct access of corticotrophins and other stimulators and inhibitors to the cell. To take advantage of this feature we have developed a method for rapidly perfusing the cells, which are embedded in a matrix of Bio-Gel beads in a small column (volume 1 ml; Lowry & McMartin, 1974). The cells are continuously perfused (1 ml/min) with Krebs-Ringer bicarbonate buffer (Umbreit et al., 1964) containing bovine serum albumin (0.5%), lima-bean trypsin inhibitor (0.05%) and glucose (0.2%).

The time-course of the steroidogenic response to ACTH (adrenocorticotrophin) was similar to that observed in vivo in sheep (Beaven et al., 1964). When an 8 min pulse of ACTH was applied there was a small but significant response at 1 min and after that the response started to build up rapidly at 4–5 min, levelling off at about 10 min. The decline of steroid output followed a similar pattern when the ACTH was withdrawn. The cells respond to physiological concentrations of natural ACTH (20–100 pg/ml) so that in this respect and also with regard to the dynamics of the steroid response isolated cells function in a similar manner to that observed in vivo.

Experiments involving the application of inhibitors to column-perfused cells show that inhibitors and products can rapidly enter and leave the cells. If aminoglutethimide is applied during stimulation with ACTH it achieves its full inhibitory effect within 1 min and the reversal of this inhibition is equally rapid. This inhibitor blocks the first step in the conversion of cholesterol into steroids so that the speed at which inhibition is manifested in addition to indicating rapid penetration into the cell also shows that no accumulation of steroid intermediates is occurring.
Perfused cells can also be used to study the stimulation of steroidogenesis by cyclic AMP and the output of cyclic AMP from adrenal cells in response to ACTH. A sub-maximal dose of cyclic AMP (0.5 mg/ml) promotes a more rapid rise in steroid output than that observed with low or high doses of ACTH. However, further investigation has shown that with larger doses of cyclic AMP (1 and 5 mg/ml) the full response builds up more slowly (Hudson & McMartin, 1974). In contrast, the shape of the onset of the response to ACTH is not very dependent on dose. Measurement of the output of cyclic AMP in response to ACTH does not help to clarify the situation because (a) large doses of ACTH are required to cause a significant output and (b) the time-course of the output varies appreciably between experiments. Although the perfused-cell system has provided clear information about these aspects of the adrenal cell, the results do not allow firm conclusions to be drawn about the role of cyclic AMP in steroidogenesis but rather serve to emphasize the complexity of this system.

Despite our lack of understanding of the processes responsible for mediating the effects of ACTH, it is possible to use isolated adrenal cells to compare the effects of different ACTH analogues on the steroidogenic process. The cell system can be used to investigate both the duration of action of different ACTH analogues and their relative potencies.

Our investigations have centred on three different peptides: corticotrophin-(1-24)-tetracosapeptide, human corticotrophin (revised sequence) and [D-Ser¹, Lys¹⁷, Lys¹⁸]-corticotrophin-(1-18)-octadecapeptide amide. In vivo the latter peptide has a longer duration of action than the other two and a greater potency (Maier et al., 1971). The perfused-cell system provides a simple test of duration of action at the cell level and when the three peptides were compared there was no significant difference in the time-course of the responses (Fig. 1). This suggests that the prolonged effects in vivo of the 1-18 peptide

![Fig. 1](image-url)  
**Fig. 1. Time-course of corticosterone output from column-perfused cells**

The cells were stimulated with a pulse of the appropriate corticotrophin during the time-interval shown by the black bar. (a) Synthetic human corticotrophin (100 pg/ml); (b) corticotrophin-(1-24)-tetracosapeptide (20 pg/ml); (c) [D-Ser¹, Lys¹⁷, Lys¹⁸]corticotrophin-(1-18)-octadecapeptide amide (100 pg/ml).
are most probably due to a difference in overall metabolic handling in the body resulting in effective plasma concentrations being maintained for longer periods of time.

Isolated adrenal cells can also be used to estimate the potencies of corticotrophins (Lowry et al., 1973). This is normally done by incubating serial dilutions of the peptide with cells for 2h and measuring the corticosterone production over this period. If this method is to give a valid comparison of the effects of the different peptides it is important to establish that extensive inactivation of the peptides does not occur during the incubation. We were able to assess this in two ways (Bennett et al., 1974), first by re-assaying the supernatant obtained after incubating cells with corticotrophins and secondly by chromatographing the products obtained from [3H-Tyr23]corticotrophin-(1–24)-tetracosapeptide (Brundish & Wade, 1973). Substantial breakdown of peptide occurred at high cell concentrations (cells from one adrenal/ml or more) but this could be greatly decreased by using dilute cell suspensions and virtually eliminated by centrifuging the cells through a cushion of 4% albumin. Interestingly the 1–24 peptide was more labile than the natural 1–39 sequence.

The fact that cell suspensions are liable to be contaminated by peptidases may go some way to explaining discrepancies in published results. For instance Seelig & Sayers (1971) found D-Ser1 N-terminal substitution greatly enhanced the potency of corticotrophins in their assay system and concluded that this could be due to aminopeptidases. In contrast, with a dilute cell suspension we have found that N-terminal substitution by D-serine has only a small effect on potency.

As shown in Fig. 2 when the isolated cell system was used to compare natural corticotrophin, corticotrophin-(1–24)-tetracosapeptide and [D-Ser1,Lys17,Lys18]corticotrophin-(1–18)-octadecapeptide amide relative molar potencies of 1:7:1.8 were observed. In vivo the first two peptides are roughly equipotent and the latter peptide is much more potent than either of the others [approx. 10 times in terms of acute effects; Maier et al. (1971)].

These results suggest that the increased potency in vivo of the 1–18 peptide is due to a difference in metabolic handling, a conclusion which is compatible with the earlier findings relating to the duration of action of the peptide.

![Fig. 2. Log dose-response curves obtained with different corticotrophins](image-url)

Steroid output was measured after a 2h incubation of the appropriate concentration of peptide hormone with isolated adrenal cells (cells from one-eighth of an adrenal/ml). ▲, Synthetic human corticotrophin; ●, corticotrophin-(1–24)-tetracosapeptide; ■, [D-Ser1,Lys17,Lys18]corticotrophin-(1–18)-octadecapeptide amide.
Fig. 3. Blood concentrations of biologically active peptide in the rat after infusion of different corticotrophins

Blood samples were collected during and after a 20 min infusion of the appropriate peptide (10 μg/min). Serial dilutions of the samples were assayed for corticotrophic activity by using the isolated-adrenal-cell assay. ▲, Synthetic human corticotrophin; ●, corticotrophin-(1-24)-tetracosapeptide; ■, [D-Ser¹, Lys¹⁷, Lys¹⁸]corticotrophin-(1-18)-octadecapeptide amide.

The difference in potencies between the 1-24 and 1-39 peptides is, however, more surprising. Since their potencies are similar in vivo it has generally been assumed that the 25-39 portion of the molecule has no influence on corticotrophic activity. The discrepancy between results in vivo and in vitro could be accounted for in two ways. Either the results in vitro do not truly represent the potency at the receptor in vivo because of damage to the receptor mechanism during isolation of the cells, or alternatively if the results in vitro are valid then there must be an appreciable difference in the metabolism of the peptides in vivo.

To clarify this situation plasma concentrations of corticotrophic activity were measured after 20 min infusions of the different peptides into rats (the species used for the bioassays in vivo and in vitro). The results (Fig. 3) showed quite clearly an appreciable difference in the handling of the peptides leading to much lower concentrations of the 1-24 than the 1-39 peptide, but the 1-18 peptide gave the highest concentrations and had a longer plasma half-life. In view of this we feel that the system in vitro probably can give a good measure of the potency of corticotrophins at the receptor level and should therefore provide a useful system for structure–activity studies. The results also show that the metabolism of corticotrophin analogues is highly dependent on their structure and plays an important part in determining the potency and duration of action in vivo.

In addition to providing a system for testing the potency of corticotrophins isolated adrenal cells also provide an opportunity to study the binding of the peptides during stimulation. In experiments with [³H-Tyr²²]corticotrophin-(1-24)-tetracosapeptide at
steroidogenic concentrations it was not possible to detect binding and we calculated that at these concentrations less than 120 receptors/cell were occupied (Bennett et al., 1974). In recent experiments binding has been detected with higher concentrations of peptide and this binding is saturated at concentrations of 0.1–1 μg of peptide/ml. This suggests that a large number of low-affinity binding sites are present and since only a small fraction can be occupied when maximum steroidogenesis is achieved it is difficult to decide whether the binding site should be identified as the receptor for the steroidogenic response. Preliminary experiments suggest that the 11–24 sequence of corticotrophin inhibits the binding in the same concentration range at which inhibition of the steroidogenic response is achieved and if this is so it suggests that the binding sites and the receptor have the same affinity for the 11–24 peptide. By experiments of this sort it may be possible to build up evidence for similarities between the binding site and the receptor.

In conclusion isolated adrenal cells appear to provide a good reflection of certain physiological processes. Studies relating to events within the cell although providing fresh information have in general served to demonstrate the complexities of the whole cell system. The cells can also be used to study the receptor properties of corticotrophins and may prove to be a useful tool for investigating structure–activity relationships.

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COMMUNICATIONS

The Assembly of Immunoglobulins

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Heavy (H) and light (L) chains of immunoglobulins are synthesized on separate polyribosomes. The completed polypeptides then assemble into the four-chain structure characteristic of immunoglobulins. This paper describes a study of the assembly process in human myeloma proteins.

Immunoglobulins of the IgG1, class were labelled with 125I and reduced in 10 mm-dithioerythritol. Heavy and light chains were separated by gel filtration in 1 m-propionic acid and dialysed against 10 mm-glycine–HCl, pH 2.5. At pH 2.5, the thiol groups

* Abbreviation: IgG, immunoglobulin G.