Calcium efflux and steroid output from superfused rat adrenal cells: effects of potassium, adrenocorticotropic hormone, 5-hydroxytryptamine, adenosine 3':5'-cyclic monophosphate and angiotensins II and III

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(Received 5 January/6 March 1981; accepted 21 May 1981)

Summary

1. The efflux of 45Ca from prelabelled dispersed rat adrenal capsular and decapsulated cell preparations was studied with a column superfusion system. Corticosterone and aldosterone outputs were measured by direct and extraction radioimmunoassays.

2. The stimulants potassium, adrenocorticotropic hormone (ACTH), serotonin and adenosine 3':5'-cyclic monophosphate (cyclic AMP), at concentrations which gave marked increases in steroid output, had no significant effect on the rate of 45Ca efflux from capsular cell preparations (mainly zona glomerulosa).

3. ACTH, at a concentration which stimulated steroidogenesis similarly, did not alter the rate of 45Ca efflux from decapsulated cell preparations (zona fasciculata/reticularis).

4. [Asp¹,Val²]Angiotensin II caused dose-dependent increases in the rate of 45Ca efflux from capsular cells which correlated with corresponding increases in steroid output, but had no effect either on 45Ca efflux or corticosterone output in decapsulated cell preparations. [desAsp¹,Ile⁵]Angiotensin II (angiotensin III) caused similar dose-dependent increases in 45Ca efflux from capsular cells, which correlated with its effects on steroidogenesis, but was less potent in both respects than angiotensin II.

5. Lowered extracellular calcium caused a very marked and rapid increase in 45Ca efflux in capsular-cell preparations, which was not significantly modified by raising the extracellular potassium concentration, although stimulation of steroidogenesis was observed.

6. These findings suggest that in zona glomerulosa cells the stimulants potassium, ACTH, serotonin and cyclic AMP are not coupled to changes in calcium transport indicated by alterations in calcium efflux, whereas angiotensins II and III, the only stimulants examined which do not increase cyclic AMP in these cell preparations, appear to act through a calcium-mediated control mechanism. In zona fasciculata/reticularis cell preparations ACTH does not appear to be coupled to such changes in calcium transport.

Key words: adenosine 3':5'-cyclic monophosphate, adrenal cortex, adrenocorticotropic hormone, aldosterone, angiotensin II, calcium metabolism, corticosterone, potassium, serotonin.

Introduction

Whereas steriodogenesis in the zona fasciculata cells of the adrenal gland is stimulated predominantly by adrenocorticotropic hormone (ACTH) and its close analogues, the zona
glomerulosa cells respond to a variety of substances [1] including ACTH, serotonin, increased extracellular potassium and angiotensin II [2] and these different types of steroidogenic responses have now been well characterized [3]. However, the details concerning the control mechanisms which link the stimulants to the steroidogenic responses still require further investigation.

In the zona fasciculata it is now generally accepted that stimulation by ACTH is mediated by adenosine 3':5'-cyclic monophosphate (cyclic AMP) [1] which acts as a secondary messenger [4, 5]. Dissociations between stimulation of corticosterone and cyclic AMP output have, however, been reported for certain fragments and derivatives of ACTH [6–9].

In zona glomerulosa cells both ACTH and serotonin have been found to increase cyclic AMP in all experiments reported [10, 11]. The data concerning stimulation by angiotensin II, however, are less consistent partly because some experimenters used high concentrations of a preparation of angiotensin II (Hypertensin), which is now known to contain an ACTH-like impurity [1]. At low concentrations of Hypertensin, stimulation of steroidogenesis has been observed without any increase in cyclic AMP [10, 12, 13]. Similarly Fujita et al. [11] showed no changes in total intracellular receptor-bound or extracellular cyclic AMP with pure angiotensin II. Only one report has so far demonstrated a correlation between cyclic AMP output and steroidogenesis with pure angiotensin II [14]. Stimulation by increasing extracellular potassium concentrations has been shown to cause increases in cyclic AMP [10, 12], although one group failed to show any increase in cyclic AMP at a concentration of potassium (15 mmol/l) which gave maximum stimulation of steroidogenesis [11]. Few data are available concerning the direct role of Ca²⁺ in the stimulation of zona glomerulosa cells. Physiological concentrations of calcium have been shown to be necessary for optimal stimulation of steroidogenesis by ACTH, potassium and angiotensin II [15] and studies on non-permissive calcium effects with calcium-transport inhibitors in adrenal capsular tissue suggest that the action of angiotensin II could be mediated by changes in calcium metabolism [13, 16].

More direct acute studies with ⁴⁵Ca-prelabelled capsular cells in static incubations have shown that stimulation of steroidogenesis by potassium (8.4 mmol/l) may involve rapid changes in calcium influx and efflux [17]. However, the techniques used in these studies are so tedious that adequate dose–response data are almost impossible to obtain. As an extension of this work we developed a dynamic superfusion system [18] based on the original method of Lowry & McMartin [19] to study directly the effects of various stimulants on ⁴⁵Ca efflux from prelabelled dispersed capsular and decapsulated cell preparations. We now describe these experiments. Part of this work has been published in a preliminary form [20, 21].

**Methods**

**Animals**

Female Sprague–Dawley rats (weighing 180–200g) were used in all experiments.

**Preparation of adrenal cell suspensions**

After adrenal decapsulation [2] cell suspensions were prepared by collagenase (Worthington Biochemical Corp., U.S.A.) digestion and mechanical dispersion of 36 adrenal capsules [22] or 18 decapsulated adrenals [23]. The cells were prepared in and finally suspended in Krebs-Ringer bicarbonate buffer ([K⁺] 3.79 mmol/l) containing glucose (2 g/l) and bovine serum albumin [Pentex fraction V: Miles Laboratories Inc., U.S.A.; 40 g/l (KRBGA medium)]. Capsular cell preparation yielded routinely 1–5 x 10⁵ zona glomerulosa cells/adrenal with 5% contamination by zona fasciculata cells, whereas decapsulated cell preparations gave routinely 2 x 10⁵ zona reticularis cells and 2 x 10⁵ zona fasciculata cells/adrenal.

**Superfusion column**

The original column superfusion method described by Lowry & McMartin [19] was found to be unsatisfactory for studies on calcium efflux due to adsorption of ⁴⁵Ca by the neoprene plunger which formed the base of the column. A polytetrafluoroethylene column was manufactured to overcome this problem and a coneshaped outlet was introduced to minimize the ‘dead’ space [18].

**⁴⁵Ca efflux experiments**

Dispersed cells suspended in 1 ml of KRBGA were prelabelled by incubation for 30 min with 10μl of ⁴⁵CaCl₂ (7 μCi) and 100 μl of [³H]inulin (1 μCi) (The Radiochemical Centre, Amersham, Bucks., U.K.) as extracellular marker to indicate the natural dead space and inertia of the system [18]. The suspension was centrifuged at 400 g for
10 min and the radioactive supernatant discarded. The cell pellet was resuspended in 2 ml of KRBGA and pumped at 2 ml/min on to a polytetrafluoroethylene column [18] containing 1 ml of Sephadex G-10 gel which had been equilibrated previously at 37°C with KRBGA for 30 min. The cells were introduced to the column through a separate inlet to avoid possible contamination with 45Ca of the superfusion medium.

Control experiments

The cells were superfused at 37°C with KRBGA ([K+] 3-79 mmol/l) for 90 min with a peristaltic pump of low pulsatile characteristics. A second peristaltic pump operating at 0.3 ml/min and connected to the main inlet tube before the join to the column served as a debubbler to prevent any air from entering the superfusion column. One minute fractions (2 ml) of superfusate were collected; 1 ml was taken for estimation of 45Ca content by liquid-scintillation counting (sufficient counts were accumulated to keep the counting error below 5%) and 1 ml was pooled into 15 min intervals up to the first 45 min for estimation of cell loss [18]. Cell losses during the first 15 min of superfusion were 15-6 (SD 5.56)% of zona glomerulosa cells in capsular cell superfusions and 40.3 (SD 13.1)% of zona reticularis and 4.23 (SD 2.19)% of zona fasciculata cells in decapsulated cell superfusions. Subsequent cell losses in both capsular and decapsulated cell experiments were less than 1% per 15 min. Steroid determinations were carried out on samples from 45 min onwards. 1 min fractions being used for direct radioimmunoassay, by using 125I-labelled indicators and a γ-counter, or 5 min pooled fractions for radioimmunoassays requiring extraction procedures, by using 3H-labelled indicators and a liquid-scintillation counter, and were expressed in ng/10⁶ cells retained by the column (after the first 45 min) per ml of superfusate. In neither case did the 45Ca contribute to the indicator counts. At the end of the superfusion perchloric acid (0-6 mol/l) was pumped through the column to lyse the cells and to release the 45Ca remaining in the cells. A 1 ml sample from the lysate (20 ml) was taken to estimate the total cellular 45Ca at 90 min.

Experiments with test solutions

After superfusion of the cells for 60 min with KRBGA control medium ([K+] 3-79 mmol/l, [Ca²⁺] 2-5 mmol/l) the test solution was superfused for 15 min followed by the control medium for the remaining 15 min. The following test solutions were used for dispersed capsular cell superfusions, with the number of experiments indicated in brackets: K⁺ 5.9 mmol/l (n = 2), K⁺ 8.4 mmol/l (n = 5), serotonin 10⁻⁴ mol/l (5-hydroxytryptamine creatine sulphate, Koch–Light Laboratories Ltd) (n = 2), ACTH 3 × 10⁻⁸ mol/l (Synacthen, Ciba–Geigy) (n = 2), [Asp¹,Val²]angiotensin II (10⁻⁹, 10⁻¹⁰ and 10⁻¹¹ mol/l; National Institute for Biological Standards and Control, Medical Research Council) (n = 2), [desAsp¹,Ile²]angiotensin II (angiotensin III; 10⁻⁸, 10⁻⁹ and 10⁻¹⁰ mol/l; Peninsula Laboratories, U.S.A.) (n = 2), cyclic AMP 4 mmol/l (Sigma (London) Chemical Co., Poole, Dorset, U.K.) (n = 2), Ca²⁺ 0.25 mol/l (n = 4) and Ca²⁺ 0.25 mol/l with K⁺ 8.4 mmol/l (n = 4). The stimulants serotonin, ACTH and angiotensins II and III were all dissolved in KRBGA control medium. Cyclic AMP was dissolved in KRBGA which was adjusted by addition of sodium hydroxide (0.6 mol/l) to maintain a pH of 7.4. In solutions containing higher [K⁺], [Na⁺] was adjusted to maintain iso-osmolarity and in the experiments where a low calcium concentration was used, iso-osmolarity was maintained by addition of sucrose (0.03 mmol/l). This concentration of sucrose had no effect either on the efflux of 45Ca or steroid output from these cells. The following test solutions were used for dispersed decapsulated cell superfusions: ACTH 3 × 10⁻⁸ mol/l (n = 5), K⁺ 8.4 mmol/l (n = 5), [Asp¹,Val²]angiotensin II (10⁻⁹ mol/l, n = 2). Cell losses, 45Ca radioactivity determinations and steroid assays were carried out as for the control experiments.

Steroid determinations

Corticosterone outputs were measured preferably to avoid studying simultaneously effects on the early biosynthetic pathway before corticosterone and the late pathway after corticosterone. We were predominantly concerned with studying effects on the early pathway in the present paper. Corticosterone was determined by direct radioimmunoassay of the superfusate with antisera raised in rabbits against corticosterone-3-mono-oxime-bovine serum albumin immunogen and 125I-labelled corticosterone-iodohistamine as ligand [24]. This assay was found to give satisfactory agreement (n = 23, r = 0.982, slope = 0.930 ± 0.022, intercept = -0.0580) with the assay previously used in our laboratories by using extraction and the hemisuccinate antibody with [³H]corticosterone as indicator [22]. Aldosterone was measured by a similar extraction method [22] for 5 min pooled samples. This
method, however, did not have sufficient sensitivity to estimate basal aldosterone outputs in experiments where \(^{45}\)Ca and corticosterone were also measured and it was therefore necessary to use the more sensitive direct radioimmunoassay as described by Al-Dujaili & Edwards [25] for these experiments, where 1 min samples were collected. In the case of aldosterone the direct assay was found to give consistently higher results than the extraction method which we think is due to the presence of an unidentified substance closely related to aldosterone in these particular cell preparations, since cross-reactivities to all major steroids are insignificant [25]. Owing to this difficulty further experiments were carried out (two for each stimulus) to check the correlation between the two assay methods. The basal and maximum aldosterone outputs for all stimulants with the direct radioimmunoassay plotted against the corresponding values obtained by the extraction method are shown in Fig. 1. Since the correlation between the two assay methods was consistent for all the stimulants examined over a wide range of values, the equation for the best-fitting straight line \(y = 1.806x - 0.00492\) was applied to adjust values obtained with the direct assay to the values which could have been expected with the extraction assay for both the non-\(^{45}\)Ca experiments (Table 1) and the \(^{45}\)Ca efflux experiments (Table 2).

**Fig. 1.** Correlation of basal and maximum aldosterone outputs obtained by direct radioimmunoassay with those obtained by extraction radioimmunoassay (●, \(K^+ 5.9\) mmol/l; O, \(K^+ 8.4\) mmol/l; ▲, ACTH \(3 \times 10^{-8}\) mol/l; Δ, serotonin \(10^{-4}\) mol/l; ■, cyclic AMP \(4 \times 10^{-3}\) mol/l; [Asp', Val']angiotensin II \(10^{-9}, 10^{-10}\) mol/l). Correlation equation: \(y = 1.806x - 0.00492\) \((r = 0.989)\).

**Table 1.** Aldosterone outputs and maximum stimulation ratios measured by extraction and direct radioimmunoassay from superfused dispersed capsular cells

<table>
<thead>
<tr>
<th>Stimulant (mol/l)</th>
<th>Basal aldosterone output (ng (10^6) cells(^{-1}) ml(^{-1}))</th>
<th>Max. S ratio for aldosterone (extraction)</th>
<th>Max. S ratio for aldosterone (corrected direct)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal aldosterone output extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(ng (10^6) cells(^{-1}) ml(^{-1}))</td>
<td>(ng (10^6) cells(^{-1}) ml(^{-1}))</td>
<td>(ng (10^6) cells(^{-1}) ml(^{-1}))</td>
</tr>
<tr>
<td>Expt. 1</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(K^+ 5.9 \times 10^{-3})</td>
<td>0.0235</td>
<td>0.0241</td>
<td>0.0198</td>
</tr>
<tr>
<td>(K^+ 8.4 \times 10^{-3})</td>
<td>0.0251</td>
<td>0.0195</td>
<td>0.0184</td>
</tr>
<tr>
<td>ACTH (3 \times 10^{-8})</td>
<td>0.0114</td>
<td>0.0195</td>
<td>0.0129</td>
</tr>
<tr>
<td>Serotonin (10^{-4})</td>
<td>0.0201</td>
<td>0.0100</td>
<td>0.0178</td>
</tr>
<tr>
<td>Cyclic AMP (4 \times 10^{-3})</td>
<td>0.0158</td>
<td>0.0125</td>
<td>0.0154</td>
</tr>
<tr>
<td>[Asp', Val']ANG II (10^{-9})</td>
<td>0.0236</td>
<td>0.0116</td>
<td>0.0237</td>
</tr>
<tr>
<td>[Asp', Val']ANG II (10^{-10})</td>
<td>0.0102</td>
<td>0.0203</td>
<td>0.0145</td>
</tr>
<tr>
<td>[Asp', Val']ANG II (10^{-11})</td>
<td>0.0262</td>
<td>0.0125</td>
<td>0.0264</td>
</tr>
</tbody>
</table>

**Results**

The efflux of \(^{45}\)Ca from both capsular and decapsulated cell preparations reached a steady state after 45 min of superfusion with a decay half-life \((t_1/2)\) of approximately 30 min, and by this time the extracellular component of \(^{45}\)Ca efflux was zero as indicated by the \(^{3}H\)inulin [18]. There was considerable variation in the initial labelling of the cells, however, this problem was
overcome to a large extent by the superfusion approach which permits the use of control data from the same experiment and allows statistical analysis to be carried out internally as well as between experiments. The first control period in all experiments with stimulants was therefore chosen from 45 to 60 min. The outputs for corticosterone (measured by the direct method) and aldosterone (measured by the extraction method) in control experiments with capsular cells were constant from 45 to 90 min with mean values of 0.931 (SD 0.235; n = 6) ng 10^6 cells^{-1} ml^{-1} and 0.0209 (SD 0.0123) ng 10^6 cells^{-1} ml^{-1} respectively. Similarly in decapsulated cell superfusions with control medium, corticosterone output remained constant from 45 min onwards with a mean value of 0.187 (SD 0.0654; n = 6) ng 10^6 cells^{-1} ml^{-1}.

Basal outputs and maximum stimulation ratios for corticosterone and aldosterone (corrected) in the 45Ca efflux experiments are shown in Table 2. In the experiments which were used to correlate the aldosterone values obtained by the direct and extraction methods, corticosterone was also measured in one experiment for each stimulant (Fig. 2).

Potassium (5.9 and 8.4 mmol/l), ACTH (3 × 10^{-8} mol/l), serotonin (10^{-4} mol/l) and cyclic AMP (4 × 10^{-3} mol/l) stimulated corticosterone and aldosterone output (Table 2), but did not have any effect on 45Ca efflux in capsular cell superfusions (Figs. 2, 3). In one experiment with serotonin some increase in 45Ca efflux was observed (Fig. 3), but statistical analysis of the appropriate five fractions after the start of the superfusion with the stimulant revealed that this was not significant (P > 0.05).

Including the data from two extra experiments with 10^{-10} mol/l angiotensin II (not included in Fig. 4 or Tables 1 and 2, because aldosterone estimates were not made), good correlation between corticosterone or 45Ca outputs and dose of angiotensin II and also between corticosterone and 45Ca output was obtained with the three doses of angiotensin II. The values for 20 collected fractions (1–20 min after the start of the infusion of stimulant) were corrected for the extrapolated baseline (steroid and log 45Ca outputs). For the log 45Ca output, the mean value for five fractions (2–6 min after the start of the infusion) was calculated. For corticosterone output, the mean value for 15 fractions (6–20 min after the start of the infusion) was calculated. These fractions included most of the stimulated outputs. By using this procedure for six control experiments with no stimulant, neither the log 45Ca nor the corticosterone mean estimates gave values significantly greater than zero.

**Angiotensin II** [10^{-11} mol/l (two experiments), 10^{-10} mol/l (4) and 10^{-9} mol/l (2)] gave outputs of 0.093 ± 0.007 (SEM), 0.71 ± 0.14 and 4.23 ± 0.85 (ng of corticosterone per 10^6 cells ml^{-1}) and 0.006 ± 0.027, 0.21 ± 0.059 and 0.31 ± 0.064 (log 45Ca c.p.m./ml) respectively. Internal testing of the data (as for the doubtful serotonin result) showed no significant increase at angio-
tensin II (10^{-11} mol/l) (two results at \( P > 0.05 \)),
but all increases at 10^{-10} mol/l (three at \( P < 0.01 \)
one at \( P < 0.05 \)) and 10^{-9} mol/l (one at \( P < 0.01 \),
one at \( P < 0.05 \)) were at least probably significant.
Spearman’s rank-correlation coefficient (between experiments) for 45Ca output
vs angiotensin II dose was 0.79 (\( P = 0.02 \)), for corticosterone output vs angiotensin II dose was
0.93 (\( P < 0.01 \)) and for corticosterone vs 45Ca output was 0.86 (\( P < 0.01 \)).

Angiotensin III (10^{-10}, 10^{-9} and 10^{-8} mol/l, two experiments for every dose) gave outputs of
0.14 ± 0.13, 1.01 ± 0.024 and 2.65 ± 0.66 (ng of corticosterone 10^6 cells^{-1} ml^{-1}) and −0.005 ± 0.020, 0.075 ± 0.013 and 0.150 ± 0.002 (log 45Ca c.p.m./ml) respectively. Internal testing of the data showed no significant increase at a concentration of angiotensin II of 10^{-10} mol/l (two results at \( P > 0.05 \)) and one significant result at 10^{-9} mol/l (one at \( P < 0.05 \), one at \( P > 0.05 \)) and two significantly increased results at 10^{-8} mol/l (two at \( P < 0.01 \)). Spearman’s rank-correlation coefficient for 45Ca output vs angiotensin III dose was 0.97 (\( P < 0.01 \)), for corticosterone output vs angiotensin III dose was 0.97 (\( P < 0.01 \)) and for corticosterone output vs 45Ca output was 0.90 (\( P < 0.05 > 0.01 \)).

Parametric analysis of the same data by using optimal transforms gave similar or slightly lower values for the corresponding probabilities.

Lowering the extracellular [Ca^{2+}] from 2.5 to 0.25 mmol/l in four experiments with capsur cells caused a pronounced increase in 45Ca efflux (Fig. 5a) with no significant effect on the basal steroid output. Increasing the potassium concentration from 3.6 to 8.5 mmol/l during the superfusion with low [Ca^{2+}] appeared to flatten the 45Ca efflux curve (Fig. 5b), but analysis of the \( t \) values for the four different components of the efflux curve, shown in Fig. 5(c), did not show this effect to be significant (Table 3).

Superfusion of decapsulated cell preparations with ACTH (3 \times 10^{-8} mol/l, \( n = 5 \)) produced a 310 (sd 51.7)-fold maximum increase in corticosterone output with no significant effect on 45Ca efflux (Fig. 6b). [Asp^1,Val^5]Angiotensin II (10^{-9} mol/l, \( n = 2 \)) produced no effect either on corticosterone output or 45Ca efflux in decapsulated cell preparations (Fig. 6a), indicating that the effect of this stimulant is specific to rat zona glomerulosa cells. Similarly, potassium at 8.5 mmol/l (\( n = 5 \)) had no effect either on corticosterone output or 45Ca efflux from superfused decapsulated cell preparations.

Discussion

Studies of the steroidogenic responses of dispersed rat adrenal capsular cells to different stimulants under static incubation conditions have demonstrated a non-linear relationship between the stimulation ratios for aldosterone and corticosterone, so that the relationship between aldosterone/corticosterone and corticosterone

![Graph](image-url)
stimulation ratios was linear and strongly correlated [3]. This relationship was much less pronounced in the superfusion system (Fig. 2a, b). In both types of experiments the slope was much less than in the static incubations and there was poor correlation between aldosterone/corticosterone and corticosterone outputs. The extraction and direct assays when carried out simultaneously, gave similar conclusions so that problems with the assay of aldosterone could not be a factor. Also the slope was not significantly different.

Fig. 3. Effect of (a) K⁺ (8·4 mmol/l, mean ± SEM, n = 5), (b) ACTH (3 × 10⁻⁸ mol/l, n = 2), (c) serotonin (10⁻⁴ mol/l, n = 2) and (d) cyclic AMP (4 × 10⁻³ mol/l, n = 2) on ⁴²Ca efflux (c.p.m./ml) and corticosterone (B) output (ng 10⁶ cells⁻¹ ml⁻¹) from superfused rat dispersed adrenal capsular cells. Where n = 2 results of individual experiments are shown. Results should be multiplied by 2·0 to convert data into c.p.m./ or ng 10⁶ cells/min.

when the zona fasciculata stimulators ACTH and cyclic AMP were excluded from the calculations, eliminating the effects of contaminating cells. These results therefore suggest that when the substrate corticosterone is rapidly removed, as in the superfusion experiments, and steroids are removed into the medium the activation effect occurring in static incubations seems to be greatly reduced. However, it could still be occurring at the biosynthetic site.

Potassium, ACTH and serotonin have all been
FIG. 4. Effect of [Asp^1,Val^1]angiotensin II (a) $10^{-9}$ mol/l ($n = 2$) (b) $10^{-10}$ mol/l ($n = 2$) and (c) $10^{-11}$ mol/l ($n = 2$) on $^{45}\text{Ca}$ efflux (c.p.m./ml) and corticosterone (B) output (ng $10^6$ cells$^{-1}$ ml$^{-1}$) from superfused rat dispersed adrenal capsular cells. Results of individual experiments are shown. Results should be multiplied by 2.0 to convert data into c.p.m./or ng $10^6$ cells/min.

FIG. 5. Effect of (a) $\text{Ca}^{2+}$ (0.25 mmol/l, $n = 4$) and (b) $\text{Ca}^{2+}$ (0.25 mmol/l) + $\text{K}^+$ (8.4 mmol/l) ($n = 4$) on $^{45}\text{Ca}$ efflux (c.p.m./ml) and corticosterone (B) output (ng $10^6$ cells$^{-1}$ ml$^{-1}$) from superfused rat dispersed adrenal capsular cells. Means ± SEM for 1 min samples ($^{45}\text{Ca}$) and 5 min samples (corticosterone) are shown. Results should be multiplied by 2.0 to convert data into c.p.m./ or ng $10^6$ cells/min. (c) Components of the $^{45}\text{Ca}$ efflux curve in response to $\text{Ca}^{2+}$ (0.25 mmol/l) for superfused dispersed capsular cells.

<table>
<thead>
<tr>
<th>$^{45}\text{Ca}$ efflux phase</th>
<th>$t_1$ $\text{Ca}^{2+}$ (0-25 mmol/l)</th>
<th>$t_1$ $\text{Ca}^{2+}$ (0-25 mmol/l) + $\text{K}^+$ (8.4 mmol/l)</th>
<th>Time interval (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.80 ± 9.50</td>
<td>17.40 ± 2.03</td>
<td>80-90</td>
</tr>
<tr>
<td>2</td>
<td>0.90 ± 0.23</td>
<td>0.71 ± 0.23</td>
<td>77-79</td>
</tr>
<tr>
<td>3</td>
<td>8.30 ± 0.25</td>
<td>14.10 ± 9.13</td>
<td>71-74</td>
</tr>
<tr>
<td>4</td>
<td>1.70 ± 0.63</td>
<td>2.40 ± 0.33</td>
<td>64-70</td>
</tr>
</tbody>
</table>
Calcium efflux from rat adrenal cells

Fig. 6. Effect of (a) [Asp\(^1\), Val\(^2\)] angiotensin II (n = 2, results of individual experiments are shown) and (b) ACTH (3 \times 10^{-8} \text{ mol/l}, mean ± SEM, n = 5) on \(^{45}\text{Ca}\) efflux (c.p.m./ml) and corticosterone (B) output (ng \(10^6\) cells\(^{-1}\) ml\(^{-1}\)) from superfused rat dispersed adrenal decapsulated cells. Data for 1 min samples (\(^{45}\text{Ca}\)) and 5 min samples (corticosterone) are shown. Results should be multiplied by 2.0 to convert data into c.p.m./ or ng \(10^6\) cells/min.

shown to increase cyclic AMP output in adrenal capsular cell preparations and in purified zona glomerulosa cells [10]. These stimulants had no effect on \(^{45}\text{Ca}\) efflux from superfused capsular cells. As expected from these negative results, cyclic AMP, which produced a dramatic increase in steroid output in the dynamic system, also failed to affect \(^{45}\text{Ca}\) efflux. These results suggest that in contrast with other cell systems described by Berridge [26] effects on calcium transport can be independent of the action of cyclic AMP in adrenal zona glomerulosa cells. In contrast with other stimulants [Asp\(^1\), Val\(^2\)] angiotensin II and angiotensin III, which were not found to alter cyclic AMP output in static incubations [27], caused significant dose-dependent increases in \(^{45}\text{Ca}\) efflux, which correlated with stimulation of steroidogenesis. This effect was specific to zona glomerulosa cells, since no effect on \(^{45}\text{Ca}\) efflux or steroid output could be detected with angiotensin II in decapsulated adrenal cell superfusions. The relative potency for angiotensin III, with respect to its effect on steroidogenesis, was 8% of that for angiotensin II, which is similar to the results obtained in static incubations [3]. Since the effects on the metabolism of the stimulant would be expected to be considerably reduced with the fast flow rate, the difference in potency between angiotensin II and III is probably more likely to be due to a difference in the intrinsic activity of the two peptides rather than a more rapid degradation of angiotensin III, which has been suggested by Catt and co-workers [28]. The data strongly suggest that angiotensins II and III act by a non-cyclic AMP-dependent mechanism in the adrenal zona glomerulosa, which involves changes in calcium transport. Other workers who reported a negative effect of angiotensin II on cyclic AMP output in zona glomerulosa tissue [13] and cells [11] have also demonstrated effects of angiotensin II on calcium transport [13, 16] by using lanthanum and verapamil, which were found to block the stimulation of aldosterone in response to angiotensin II by inhibiting calcium transport.

Mackie et al. [17] reported that incubation of \(^{45}\text{Ca}\)-prelabelled capsular cells with 8.4 mmol/l concentration of potassium caused a greater retention of \(^{45}\text{Ca}\) than incubation with 3.6 mmol of potassium/l. Separation of cells from the medium was carried out by centrifugation through phthalate ester oil. Based on the rapidity of effect on efflux, and the lack of effect of potassium (8.4 mmol/l) on \(^{45}\text{Ca}\) uptake, on total intracellular calcium concentration and on total intracellular potassium concentration [29], they suggested that the primary effect of potassium on zona glomerulosa cells was either to alter the rate of release of calcium from a loosely bound exchangeable pool (i.e. a fast compartment) or on the redistribution of calcium within the cell. In the superfusion experiments described it is not possible to study the rapid effects reported by Mackie et al. [29], since the efflux of extracellular \(^{45}\text{Ca}\) (remaining after prelabelling the cells) has a similar \(t_1\) to that of the fast cellular compartment. However, lowering the extracellular calcium concentration from 2.5 to 0.25 mmol/l after 60 min of superfusion caused a very rapid increase in \(^{45}\text{Ca}\) efflux which had a \(t_1\)
comparable with that for the fast compartment. No reproducible effect of a 8-4 mmol/l concentration of potassium was detected on $^{45}$Ca efflux from this fast compartment in four experiments, although stimulation of steroidogenesis was observed. It is possible that there was a small effect which could be revealed by further experimentation if this were possible or that this fast compartment may not represent $^{45}$Ca efflux from the same calcium pool studied in experiments by Mackie et al. [29]. Although the correlation between corticosterone and $^{45}$Ca output values is good, as detailed in the Results section, it is impossible to believe that the important physiological signal for the simulation of steroidogenesis is calcium efflux. It is more likely that this effect is related to another critical quantity such as intracellular ionized calcium concentration which presently cannot be measured in such small cells as the zona glomerulosa cells without significant perturbation of normal processes. More direct investigations must await the availability of a suitable calcium probe [30].

The role of calcium as the mediator in the mechanisms of action for angiotensin II does not appear to be restricted to the adrenal gland. Effects of angiotensin II on carbohydrate metabolism in hepatocytes have been shown to be independent of cyclic AMP and to involve changes in calcium transport [31, 32]. Similarly, the contractor response of smooth muscle to angiotensin II and the inhibition of renin release by angiotensin II in the isolated perfused kidney both appear to be mediated by similar calcium-dependent mechanisms [33].

Acknowledgments

This work was supported by a Medical Research Council grant (G969204C). We thank Dr C. R. W. Edwards and Dr. E. Al-Dujaili, St Bartholomew's Hospital, London for providing antisera and $^{125}$I-labelled indicator for the direct assay of aldosterone and for collaboration in development of the direct radioimmunoassay for corticosterone. We are also very grateful to F. A. F. Zananiri for invaluable technical help, Mrs M. Stuart for typing the manuscript and S. Nightingale for audiovisual assistance. Dr Janet Bell assisted in the modification of the final paper.

References

Calcium efflux from rat adrenal cells


