ATP, CALCIUM UPTAKE AND GROWTH HORMONE RELEASE

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1. Introduction

The rate of growth hormone release from anterior pituitaries incubated in vitro can be increased by increasing the extracellular potassium ion concentration in the presence of calcium ions [1–3]. The rates of release of TSH [4], FSH and LH [5], and ACTH [6, 7] are also increased with increasing potassium ion concentration, but the rate of prolactin release is unchanged [6]. An increase in the extracellular potassium ion concentration has been shown to reverse the membrane potential of a proportion of anterior pituitary cells [7], and it is possible that this results in an increased influx of calcium raising the cytoplasmic free calcium concentration and hence triggering release. The stimulation of growth hormone release by high potassium concentrations is inhibited by CCCP (trichloromethoxycarbonylcyanide phenylhydrazone), an uncoupler of oxidative phosphorylation [1]. It is possible that this requirement of release for ATP is due to the direct involvement of ATP in the release process, although it might be mediated by an ATP involvement in calcium entry. We have therefore investigated the quantitative relationship between ATP concentration, growth hormone release and calcium incorporation into pituitary slices using various concentrations of 2,4-dinitrophenol and CCCP (uncouplers of oxidative phosphorylation), and rotenone (a respiratory chain inhibitor) to produce a range of ATP values.

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2. Methods

The incubation system employed has been described elsewhere [8] and need not be presented in detail here. Pituitary slices obtained from heffers were preincubated for 30 min at 37° in 3 ml Krebs–Henseleit bicarbonate buffered salt solution with or without uncoupler or inhibitor. They were then blotted, weighed and incubated at 37° for 60 min in 3 ml of either the normal medium or a high potassium medium in which the potassium concentration had been raised from 5.9 to 65 mM and the sodium concentration reduced from 143 to 84 mM, and which contained uncoupler or inhibitor at the same concentration as that used in the preincubation. All media were supplemented with 2.5 mM glucose, sodium glutamate and sodium hydroxybutyrate.

At the end of incubation, the slices were transferred to 1 ml of cold 5 percent PCA, agitated on a vortex mixer and homogenised. The supernatant was neutralised with KOH–ethanolamine and the ATP in the supernatant measured in a fluorimeter using hexokinase and glucose-6-phosphate dehydrogenase. The growth hormone concentration in the medium was measured using a double antibody radioimmunoassay [8].

Calcium incorporation was assessed in a parallel series of experiments in which slices, preincubated in the presence of uncoupler or inhibitor as before, were incubated for 90 min in normal or high potassium medium containing ^{45}Ca (1 μCi/ml) in addition to the uncoupler or inhibitor. At the end of the incubation the slices were blotted and transferred through four successive washes in 3 ml normal medium, each wash

Table 1
Effect of increased extracellular potassium concentration on growth hormone release, pituitary ATP concentration, and ^{45}Ca-calcium incorporation.

	Composition of medium	
	Na : 143.1 mM K^+ : 5.9 mM	Na 84 mM K 65 mM
GH release rate (μg/mg wet/hr)	1.36 ± 0.15	5.63 ± 0.37
ATP concentration (μmoles/g wet)	0.87 ± 0.04	0.88 ± 0.04
^{45}Ca-calcium incorporation* (cpm/mg wet/90 min)	459 ± 22	634 ± 20

Data represent mean (± standard error of mean) for 40 slices.
* Medium specific activity = 3.94×10^5 cpm/μmole calcium.

taking about 5 min, to remove extracellular calcium. They were then blotted, weighed, and digested for 18 hr at 45° with shaking in 0.4 ml Nuclear Chicago Solubiliser (a solution of quaternary amines in toluene [9]). The ^{45}Ca incorporated into the slice was determined in a liquid scintillation counter.

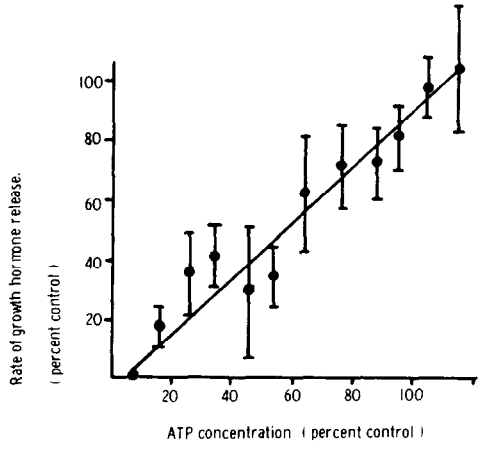

Fig. 1. Influence of pituitary ATP concentration in the increase in growth hormone output caused by high potassium media. Each point represents the mean ± the standard error of the mean for slices whose ATP concentrations fell within a 10 percent range. Rotenone, CCP or 2.4 DNP were used to reduce the ATP concentrations.

3. Results

a) The effect of an increased extracellular potassium concentration on ^{45}Ca-calcium incorporation, the rate of growth hormone release, and the ATP level is given in table 1. Incorporation of ^{45}Ca-calcium was increased by 38 percent, the rate of growth hormone release was increased fourfold, while the ATP level was unchanged. The increase in calcium incorporation represents 0.44 nmole calcium at the specific activity of the medium, and as this is low compared to the intracellular calcium content of 8.44 ± 0.85 nmoles/mg (wet) [10] it is not possible to decide whether it represents a net influx of calcium ions, or calcium exchange.

b) The effect of DNP, CCCP, and rotenone on growth hormone release and pituitary ATP concentration is shown in fig. 1. The data in this figure were obtained by calculating the ATP concentration and potassium induced increase in growth hormone output for each slice, as a percentage of the control for that experiment. The data obtained in this way from four experiments were combined to give the mean increase in growth hormone release (± standard error) for slices whose ATP concentrations fell within successive 10 percent increments. It can be seen that the increase in the rate of growth hormone release was linearly to the ATP concentration over the range of ATP values obtained.

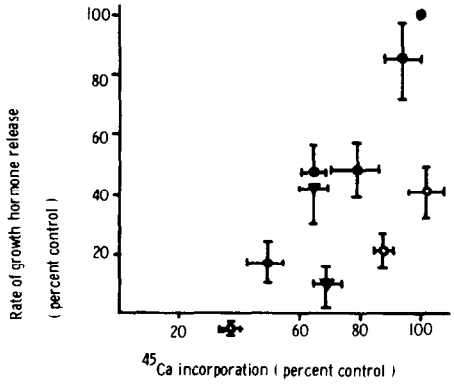

Fig. 2. Variation in the increase in growth hormone output and ^{45}Ca-calcium incorporation into slices. The slices were incubated with rotenone (▾) at concentrations of 0.2 or 1.0 μg/ml, with CCCP (●) at concentrations of 0.4, 0.2, 0.15, and 0.05 μg/ml, or with 2,4-DNP (○) at concentrations of 0.5, 0.1, 0.05 and 0.01 mM. The bars represent the standard error of the mean of at least eight slices.

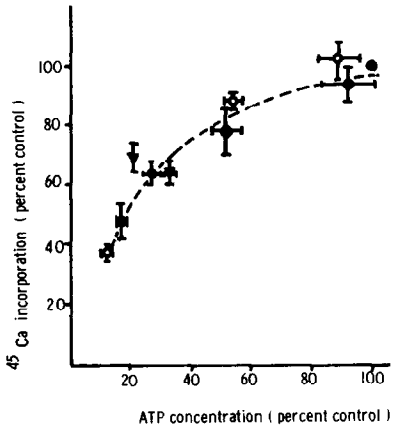

Fig. 3. Influence of pituitary ATP concentration on ^{45}Ca-calcium incorporation into slices. For legend see fig. 2.

c) Fig. 2 shows the relationship between the increase in the rate of growth hormone release and calcium incorporation. In this figure the mean percent increase in growth hormone release observed at a given concentration of DNP, CCCP or rotenone was plotted against the mean percent calcium incorporation observed in parallel experiments at the same concentration of uncoupler or inhibitor. The data appear to demonstrate that stimulation of growth hormone output is observed only when the calcium incorporation exceeds a threshold value. Fig. 3 shows the relationship between calcium incorporation and ATP concentration, plotted in the same way as fig. 2. The incorporation of calcium and the ATP concentration are reduced together, apparently in a non-linear fashion. The dependence of ^{45}Ca incorporation on ATP concentration was an unexpected finding and may possibly reflect an ATP dependent calcium uptake into the cell, although alternative explanations such as decreased rate of exchange or an ATP dependent calcium entry into a subcellular fraction are also possible. At the highest concentration of CCCP used (0.4 μg/ml) the intracellular calcium content of the slice was 7.25 ± 1.18 nmoles/mg (wet), which did not differ significantly from control values [10], but this does not preclude the possibility that the exchangeable calcium pool is small and that it falls when the ATP level is reduced.

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