STIMULATION BY THYROLIBERIN (TRH) OF ^{86}Rb EFFLUX FROM PERIFUSED BOVINE ANTERIOR PITUITARY CELLS

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1. Introduction

Activation of receptors linked to phosphatidylinositol turnover is frequently associated with increases in cyclic GMP content and potassium efflux in the bovine anterior pituitary [1]. Acetylcholine, which stimulates growth hormone secretion, increases pituitary cyclic GMP concentrations, phosphatidylinositol labelling, and ^{86}Rb efflux, in vitro [2,3]. Some of the hypothalamic peptides which regulate anterior pituitary hormone secretion may also elicit this pattern of responses. Thyroliberin (TRH) stimulates the secretion of prolactin and thyrotropin in many species although its effects on growth hormone secretion are controversial [4]. Since TRH increases pituitary cyclic GMP concentrations [5,6] and the incorporation of phosphate into pituitary phospholipids [7,8], it might stimulate potassium efflux from pituitary cells.

Pituitary cells are electrically active and TRH increases the frequency of the action potentials [9,10]. Increased calcium entry through a channel opened during the depolarising phase of the action potential may raise the cytoplasmic calcium concentration and stimulate secretion [11]. Tetraethylammonium ions (TEA) delay the repolarising phase of the action potential, presumably by inhibiting outward potassium movement [12]. If the action potentials are related to hormone secretion, TEA should potentiate TRH-induced secretion.

We report here that TRH increases the efflux from perifused pituitary cells of ^{86}Rb, whose cellular transport mimics that of potassium [13], and causes a burst of prolactin secretion without greatly affecting growth hormone secretion. In the presence of TEA, growth hormone secretion induced by TRH was markedly potentiated, prolactin secretion was not affected, and ^{86}Rb efflux was slightly inhibited. The maximal rate of ^{86}Rb efflux in response to TRH coincided with the falling phase of hormone secretion. The data suggest that changes in potassium permeability regulate the secretory responses in the anterior pituitary.

2. Materials and methods

2.1. *Preparation and perifusion of pituitary cells*

Bovine anterior pituitary glands (obtained from heifers) were dispersed using collagenase in the presence of soyabean trypsin inhibitor, and a fraction enriched in lactotrophs and somatotrophs obtained by centrifugation through Percoll, as in [3]. The cells (2×10^7) were incubated for 90 min in 1 ml medium containing ^{86}Rb (100 μCi, 50 μM), NaCl (118 μM), KCl (5.9 mM), KH_2PO_4 (1.2 mM), $MgCl_2$ (1.2 mM), $CaCl_2$ (2.5 mM), $NaHCO_3$ (21 mM), sodium 3-hydroxybutyrate (1.2 mM), glucose (2.8 mM) and bovine serum albumin (Sigma, fraction V, 1 mg/ml), and equilibrated with O_2:CO_2 (95:5). The cells were then resuspended in the same medium containing no rubidium, and aliquots containing 2.5×10^6 cells were transferred to 8 columns each containing 0.4 ml swollen Sephadex G-10. The columns were perifused with the same medium at 37°C and 0.2 ml/min flowrate, collecting 2 min fractions. In each experiment TEA (24 mM) was introduced into 2 channels after 52 min, and TRH (10^{-7} M) was introduced into these channels in the presence, and into 2 parallel channels in the absence, of TEA after 66 min.

2.2. *Assay procedures*

Growth hormone and prolactin were assayed by radioimmunoassay, the prolactin assay using a rabbit

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antiserum to ovine prolactin (NIH, P-S11). Cross-reactions of prolactin and thyrotropin in the growth hormone assay were 0.15% and 0.05%, and of growth hormone and thyrotropin in the prolactin assay were 0.27% and 0.02%, respectively. These data were obtained in 5 expt. using 5 different cell prepn. Since the absolute rates of hormone release varied between experiments, the release for each channel was normalised to bring the average rate of release during the control period (40–52 min) for that channel to 100. The ^{86}Rb content of the fractions was determined by Cerenkov counting, and normalised by calculating the fractional efflux rate [3].

3. Results

Fig.1 shows the effects of TRH on ^{86}Rb efflux and prolactin secretion from bovine pituitary cells perifused in the presence and absence of TEA. TRH increased the fractional efflux of ^{86}Rb by ~2.5-fold max; the efflux took 4 min to reach its peak and was sustained for 6 min at approximately the maximal

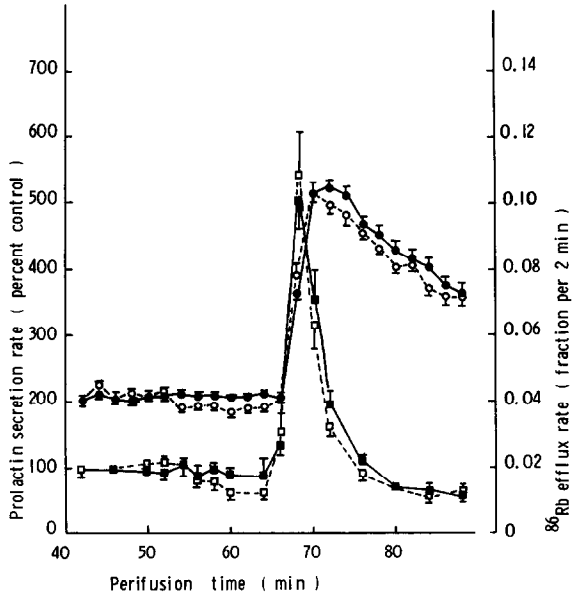

Fig.1. Stimulation of ^{86}Rb efflux and prolactin secretion by TRH. The data are the average ^{86}Rb efflux (●,○) and prolactin secretion (■,□) from duplicate columns from 5 batches of pituitary cells. TEA (24 mM) was present from 52 min (□,○); TRH (10^{-7} M) was present from 66 min in the absence (■,●) or presence (□,○) of TEA (24 mM). Vertical bars show 1 SEM.

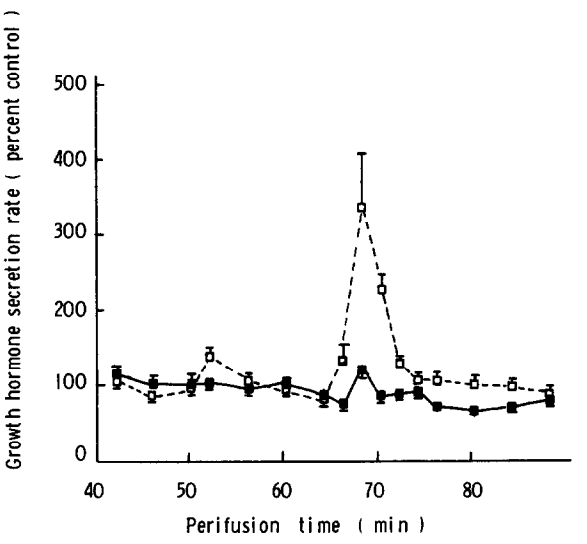

Fig.2. Potentiation of TRH-induced growth hormone secretion by TEA. The data are the average growth hormone secretion, in the absence (■) or presence (□) of TEA and subsequently TRH, for the samples shown in fig.1.

rate before falling gradually. TRH also caused a burst of prolactin secretion, which was maximal after 2 min and then fell sharply reaching the basal level after ~10 min. There was only a minor change in growth hormone secretion after TRH additional (fig.2).

The addition of TEA (24 mM) only slightly decreased basal ^{86}Rb efflux from the cells. It did not delay the rising phase of efflux seen when TRH was subsequently added, and again only slightly decreased the peak efflux (fig.1). TEA did not modify the secretion of prolactin in response to TRH (fig.1). However, TEA caused a small increase in growth hormone secretion, and subsequently it markedly potentiated the secretion of growth hormone in response to TRH (fig.2). The maximum rate of growth hormone secretion was seen after 2 min, but although the rate then fell it was still above the level caused by TRH alone after 14 min.

4. Discussion

The data presented here show that TRH, like acetylcholine [3], increases ^{86}Rb efflux from perifused bovine pituitary cells. Potassium efflux responses are well characterised in salivary glands [1], but in the anterior pituitary the mechanisms of the efflux and

its possible relationship to hormone secretion are not known. The time courses of the two responses and the effect of TEA on them may be relevant to these questions.

In anterior pituitary cells, TEA delays the repolarising phase of the action potential [12], presumably by inhibiting a potential-sensitive potassium channel as it does in other cell types [14]. The ability of TEA to potentiate TRH-induced growth hormone secretion provides indirect evidence that potassium efflux through this channel limits the secretory response of somatotrophs to TRH. Possibly TEA prolongs the period of calcium entry during TRH-induced action potentials, by delaying repolarisation, and the cytoplasmic calcium concentration therefore reaches a level capable of stimulating growth hormone secretion. The fact that TEA potentiated growth hormone secretion without greatly inhibiting ^{86}Rb efflux might suggest that only a small proportion of the total ^{86}Rb efflux uses this potential-sensitive channel. Although this may in fact be so, the rise in cytoplasmic calcium which leads to increased growth hormone secretion in the presence of TEA and TRH could also activate a calcium-dependent potassium channel, which would not be inhibited by TEA [14]. Efflux of ^{86}Rb might therefore still be stimulated by TRH in the presence of TEA, but might use a different channel. The effect of TEA prompts 2 other comments:

(i) Since freshly dispersed bovine somatotrophs can respond to TRH in the presence of TEA they presumably possess TRH receptors, which may be relevant to the physiological regulation of growth hormone secretion.

(ii) Lactotrophs appear to lack a functional potential-sensitive potassium channel since TEA did not potentiate prolactin secretion in response to TRH.

We have also found that TEA and another inhibitor of potassium channels, 4-aminopyridine, potentiate secretion of growth hormone but not prolactin in response to acetylcholine ([3], unpublished). This could be causally related to the high basal prolactin secretion observed in vitro [15].

The second point to emerge from the data was the difference between the time courses of ^{86}Rb efflux and hormone secretion, which meant that the maximum ^{86}Rb efflux was observed while secretion was falling rapidly. A similar relationship between the maximal rate of ^{86}Rb efflux and the falling phase of growth hormone secretion was observed after exposure of pituitary cells to acetylcholine [3]. The difference in time courses of secretion and of ^{86}Rb efflux could arise if the 2 processes differ in their coupling to receptor activation or in their methods of termination. Alternatively, it could occur if part of the ^{86}Rb efflux were associated with processes which terminate the secretory response. Efflux of ^{45}Ca from pituitary cells exposed to veratridine, which activates sodium channels in excitable cells, was also delayed relative to growth hormone secretion [16]. Possibly the termination of secretion involves a process in which calcium and potassium are lost from the cells.

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