Inhibition by dopamine of ^{86}Rb efflux and hormone secretion from bovine anterior pituitary cells perifused in the presence of acetylcholine or TRH

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The effects of dopamine (10^{-5} M) on growth hormone and prolactin secretion and the fractional rate of ^{86}Rb efflux were investigated using perifused bovine pituitary cells. Dopamine decreased the control efflux rate, prevented the rise caused by thyroliberin (10^{-7} M) in the presence of 3-isobutyl-1-methylxanthine (10^{-4} M) and greatly decreased the rise caused by acetylcholine (2.5×10^{-5} M). Under these conditions it inhibited the prolactin secretion but it had no effect on growth hormone secretion induced by either treatment. Dopamine receptors in lactotrophs are therefore coupled to secretion and potassium permeability, but if somatotrophs contain dopamine receptors they are coupled only to K^+ efflux.

Prolactin *Growth hormone* *Thyroliberin* *Dopamine* *Anterior pituitary* *Potassium permeability*

1. INTRODUCTION

We have shown that the hypothalamic peptide TRH (thyroliberin) stimulates the secretion of prolactin and the efflux of ^{86}Rb, an isotope which behaves like potassium, from perifused bovine pituitary cells in vitro [1] and that acetylcholine increases growth hormone release and ^{86}Rb efflux from these cells [2]. Anterior pituitary cells exhibit spontaneous and evoked action potentials, and it is possible that calcium entry during the action potentials leads to secretion since for example TRH increases their frequency [3]. Our data [1,2] suggest that there is a connection between potassium permeability, electrical activity and growth hormone secretion from somatotrophs. Thus 4-aminopyridine and tetraethylammonium ions, which are potassium channel blockers [4] and increase the frequency [5] and duration [6], respectively, of action potentials in pituitary cells, potentiate the secretion of growth hormone caused by TRH [1] and acetylcholine [2].

Dopamine inhibits prolactin secretion in vitro from normal and tumour pituitary cell lines [7], and can also inhibit growth hormone release from normal and adenomatous human pituitaries [7]. Since dopamine decreases the electrical activity of pituitary cells [8], the opposite effect to that of TRH, it is of interest to determine whether dopamine also has the opposite effect on ^{86}Rb efflux. Therefore, we have investigated the effects of dopamine on growth hormone and prolactin secretion and ^{86}Rb efflux from perifused bovine pituitary cells. We report here that dopamine inhibited the basal fractional ^{86}Rb efflux rate, prevented the rise in efflux caused by TRH in the presence of 3-isobutyl-1-methylxanthine (IBMX), and greatly decreased the rise in efflux caused by acetylcholine. Dopamine also inhibited prolactin secretion caused by TRH in the presence of IBMX and by acetylcholine, but had no effect on the growth hormone secretion induced by either treatment. Dopamine receptors in lactotrophs may therefore be coupled to secretion and potassium permeability, but if somatotrophs contain dopamine receptors they are only coupled to potassium efflux.

2. MATERIALS AND METHODS

The experimental procedures were as outlined in [1]. Bovine anterior pituitary glands were dispersed using collagenase (Boehringer; 1 mg/ml) in the presence of soyabean trypsin inhibitor (Sigma; 0.3 mg/ml), and a fraction enriched in lactotrophs

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and somatotrophs obtained by centrifugation through Percoll. The cells (2×10^7) were incubated for 90 min in 1.5 ml medium containing: ^{86}Rb (100 μCi, 50 μM), NaCl (118 mM), 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, v/v). The cells were then suspended 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 a flow rate of 0.2 ml/min, collecting 2 min fractions. In each experiment 4 sets of conditions were performed in duplicate. The first 20 fractions (40 min) were discarded and the next 24 fractions were collected. Dopamine (10^{-5} M) or IBMX (10^{-4} M) were introduced after 50 min and TRH (10^{-7} M) or acetylcholine (2.5×10^{-5} M) after 64 min, were appropriate.

The concentrations of growth hormone and prolactin were determined by radioimmunoassay [1], and in order to combine the data from a number of experiments using different cell preparations, the hormone release in each channel was normalised to bring the mean rate of release during the control period (41–50 min) for that channel to 1. The ^{86}Rb content of the fractions was determined by Cerenkov counting and normalised by expressing the efflux as the fraction of the total ^{86}Rb present in the cells at the beginning of each 2 min period which was washed out during that period.

3. RESULTS

The effects of dopamine on the responses to acetylcholine are shown in fig.1. Dopamine decreased the basal fractional rate of ^{86}Rb efflux and almost completely prevented the subsequent rise in efflux caused by acetylcholine (fig.1A). It had no effect on the secretion of growth hormone caused by acetylcholine (fig.1B), but decreased basal prolactin secretion and prevented the small rise caused by acetylcholine (fig.1C).

The effects of TRH were examined in the presence of IBMX (10^{-4} M) because this methylxanthine potentiates the increases in prolactin release and cyclic AMP concentration caused by TRH [9] and because we have found that it also potentiates TRH-induced growth hormone secretion (unpublished). IBMX itself increased the frac-

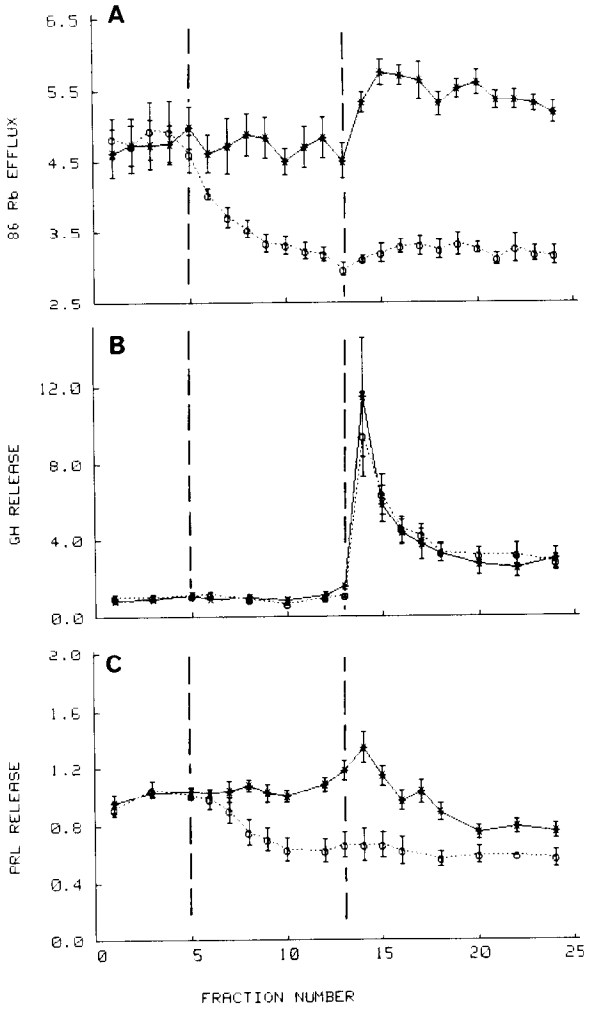

Fig.1. The figure shows the fractional rate of ^{86}Rb efflux (A), the rate of growth hormone secretion (B) and the rate of prolactin secretion (C) from perifused bovine pituitary cells. The first 20 two min fractions were discarded and fraction 1 in the figure therefore refers to material released in the 41st and 42nd min of perifusion. Dopamine (10^{-5} M) was present in one channel (...o...) from the 50th min (fraction 6) onwards, and acetylcholine (2.5×10^{-5} M) was present in both channels from the 64th min (fraction 13) onward. Data are means and bars standard errors for 6 columns from 3 cell batches; other details are as in section 2.

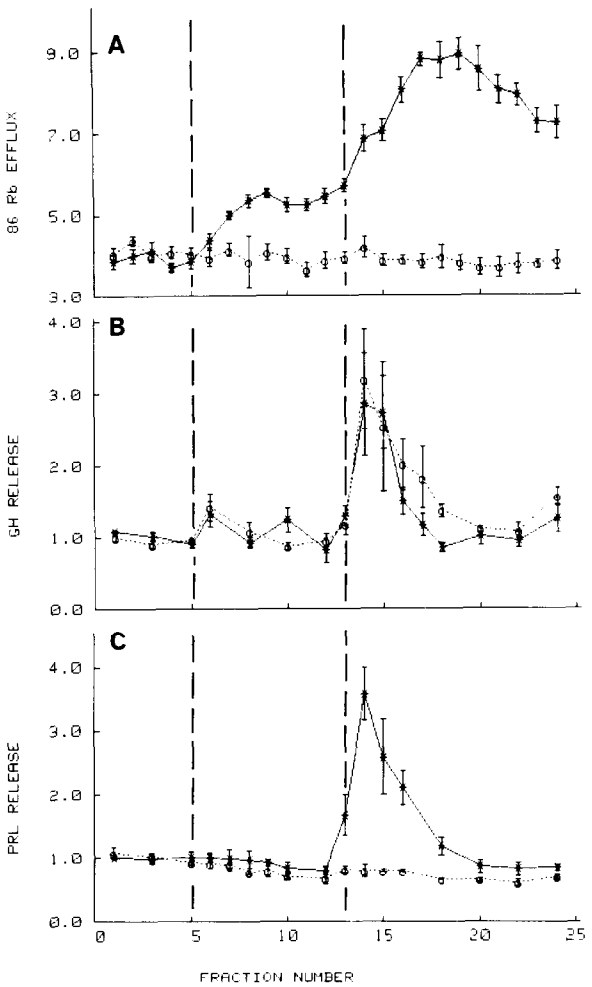

Fig.2. Dopamine (10^{-5} M) present in one channel (...o...) from the 50th min (fraction 6) onwards; IBMX (10^{-4} M) was present in both channels from the 50th min (fraction 6) onwards and TRH (10^{-7} M) was present in both channels from the 64th min (fraction 13) onwards; other details are as in fig.1 and section 2.

tional rate of ^{86}Rb efflux and when TRH was added there was a further large increase in ^{86}Rb efflux (fig.2A). When dopamine was added with IBMX it prevented the rises in efflux caused by both the methylxanthine and by TRH, but did not decrease basal efflux (fig.2A). IBMX alone had little effect on prolactin or growth hormone secretion but in its presence TRH increased both growth hormone (fig.2B) and prolactin (fig.2C) secretion. When dopamine was added with IBMX, it prevented prolactin (fig.2C) but not growth hormone (fig.2B) secretion in response to TRH.

4. DISCUSSION

These data show that dopamine inhibits prolactin but not growth hormone secretion from bovine pituitary cells and that it inhibits all of the ^{86}Rb efflux caused by TRH and most of that caused by acetylcholine. Taken at face value the implications are that dopamine receptors are only present on bovine lactotrophs and that most of the ^{86}Rb efflux comes from them. Data on the presence of dopamine receptors on somatotrophs from other species are conflicting, but dopamine inhibits growth hormone release from human pituitaries in vitro [7,10] and dopamine receptors have been demonstrated by light microscopy on rat somatotrophs [11] even though dopamine apparently does not inhibit rat growth hormone secretion in vitro [12]. Therefore, it is possible that dopamine receptors are usually present on somatotrophs, but that they are not always coupled to the inhibition of growth hormone secretion.

Whether or not this is so, it is clearly possible to stimulate growth hormone release in vitro without a large associated increase in ^{86}Rb efflux. If the secretion of growth hormone involves increased electrical activity, as the potentiation of secretion by 4-aminopyridine and tetraethylammonium would seem to indicate [1,2], then it follows that dopamine does not prevent the increase in somatotrophs and that very little ^{86}Rb efflux needs to occur during it. Moreover, dopamine did not affect the termination of the burst of growth hormone secretion even though it inhibited the majority of the ^{86}Rb efflux caused by TRH in the presence of IBMX or by acetylcholine. Therefore, it is unlikely that the termination of the secretory episodes is brought about by increased potassium efflux, perhaps through calcium-activated potassium channels [4], hyperpolarising the somatotroph plasma membrane and terminating calcium entry. The data strongly suggest that the majority of the observed ^{86}Rb efflux is not tightly coupled to either the initiation or termination of growth hormone secretion.

The mechanism whereby dopamine inhibits ^{86}Rb efflux could be related to the mechanism by which it inhibits prolactin release, for which there

are several proposals. One proposal is that dopamine decreases the pituitary cyclic AMP concentrations by inhibiting adenylate cyclase and that this inhibits prolactin release [13,14]. The effects of dopamine and IBMX on the fractional rate of ^{86}Rb efflux could be related to changes in cyclic AMP metabolism. If dopamine inhibited cyclic AMP synthesis and IBMX, by inhibiting cyclic AMP phosphodiesterase, inhibited cyclic AMP breakdown [9], their combined actions on the concentration of cyclic AMP might, like their combined actions on ^{86}Rb efflux, cancel each other out. An alternative proposal, supported by the ability of the ionophore A23187 to overcome dopamine inhibition of prolactin release [15], is that dopamine inhibits calcium entry into lactotrophs. The inhibition could be secondary to an alteration in chloride permeability affecting the functioning of the calcium channel as suggested in [8]. Inhibition of calcium entry might also explain the decrease in potassium efflux since a fall in the cytoplasmic calcium concentration could cause the activity of the calcium-sensitive potassium channel to drop [4]. This would explain the data satisfactorily provided all the ^{86}Rb efflux in the presence of TRH and IBMX, and most of the efflux in the presence of acetylcholine come from lactotrophs. However, dopamine cannot prevent a rise in cytoplasmic calcium in somatotrophs since it did not inhibit growth hormone secretion. Therefore if some of the ^{86}Rb efflux comes from somatotrophs, the inhibition of that efflux by dopamine cannot be explained in terms of decreased calcium concentration. The mechanism by which dopamine inhibits ^{86}Rb efflux, like the mechanism by which it inhibits prolactin release, remains to be established.

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