DOPAMINERGIC CONTROL OF TSH SECRETION IN ISOLATED RAT PITUITARY CELLS

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1. Introduction

There is now considerable evidence to indicate that dopamine has an inhibitory role in the control of TSH secretion in man [1]. Studies with the dopamine antagonist domperidone [2–4], which does not cross the blood brain barrier [5] suggest that this dopaminergic control is exerted at the level of the anterior pituitary or median eminence [6,7].

This report describes an investigation of the effects of dopamine on the secretion of TSH by isolated rat pituitary cells. Our studies demonstrate that dopamine can inhibit TSH secretion directly and suggest that this type of interaction could be involved in the control of TSH secretion in vivo.

2. Methods

Rat pituitary cells were isolated by minor modifications the methods in [8–10]. Adult male Porton-Wistar rats (180–220 g) were used throughout and the anterior pituitary glands were removed into 20 ml of Ca^{2+}- and Mg^{2+}-free Earles salts (Gibco) (at 4°C) and supplemented with 100 U penicillin, 100 mcg streptomycin and 0.25 mcg Fungizone. The glands were washed 4 times in this solution before being cut into small pieces and dispersed in 5 ml of 3% bovine serum albumin (BSA fraction V, Sigma), 0.4% collagenase (type I, Sigma), 0.2% Dispase II (Boerhinger, Mannheim), 0.1% hyaluronidase (Type S, Sigma), 0.01% deoxyribonuclease (DNase I, Sigma), in 137 mM NaCl; 5 mM KCl; 0.7 mM Na_2HPO_4; 10 mM glucose; 25 mM Hepes for 75–90 min at 37°C. Further dispersion was achieved by gentle repeated suction through a siliconised Pasteur pipette.

The cells were washed 3 times in alpha-modified minimum essential medium (α-MEM, Flow) supplemented with antibiotics (as above), 10% fetal calf serum (Gibco) and 1 mM glutamine, resuspended in the same medium and seeded in 1 ml aliquots ($0.25–0.5 \times 10^6$ cells/ml) into multiwell plates (Falcon 3008).

The cells were then cultured in an atmosphere of water-saturated 95% air, 5% CO_2, at 37°C and at 2 days the cultures were adjusted to 0.5 mM in dibutyryl cAMP (Sigma).

Experiments were performed on the third day of culture, the medium being replaced with α-MEM alone and the appropriate test material added in 20 μl aliquots 6–10 wells/test point. Significance was determined by Student's *t*-test. The cells were incubated for 3 h at 37°C after which time the medium was removed, diluted with an equal volume of BSA (1% in 0.01 M PO_4; 0.15 M NaCl; 0.01% merthiolate), stored at −20°C and then assayed for TSH and prolactin using radioimmunoassay materials kindly provided by Dr A. F. Parlow of the National Pituitary Agency. TSH measurements were expressed as ng NIAMD-rat TSH-RP-1.

3. Results

Thyrotrophin releasing hormone (TRH; Roche) at 10^{-10}–10^{-7} M stimulated TSH secretion by the pituitary cells in a dose-dependant manner. For studies on the effects of dopamine on TRH-stimulated TSH secretion a concentration of TRH (5×10^{-8} M) that gave submaximal stimulation was used. Similarly a submaximal concentration (5×10^{-4} M) of dibutyryl cyclic AMP (DBC, Sigma) was also used in studies of the effects of dopamine on DBC-stimulated TSH secretion.

Dopamine was found to inhibit secretion of TSH by both TRH stimulated and non-stimulated cells.

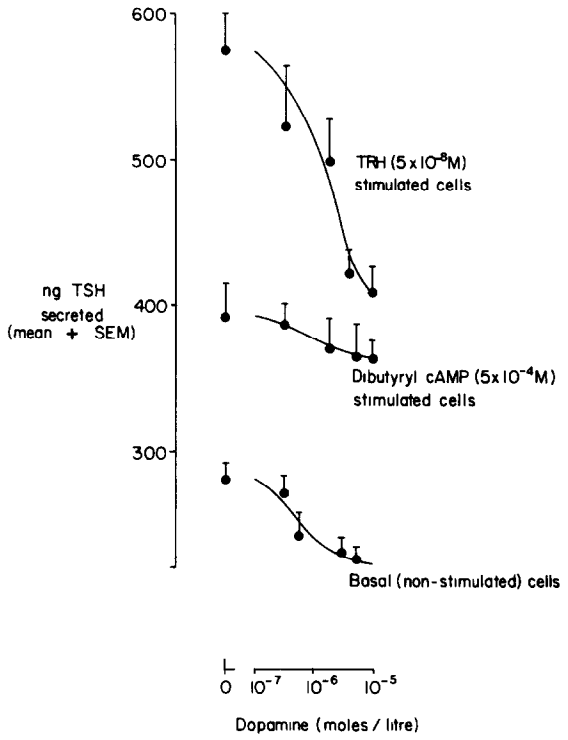

Fig.1. Direct inhibition of TSH secretion by dopamine.

The effect was dose-dependant over 10^{-7}–10^{-5} M with significant inhibition ($P < 0.01$) of secretion occuring at 10^{-6} M (fig.1). In contrast to its effects on non-stimulated and TSH-stimulated cells, dopamine did not significantly inhibit TSH secretion by DBC-stimulated cells (fig.1).

TSH secretion by non-stimulated pituitary cells could also be inhibited in a dose-dependant manner by the dopamine agonist bromocryptine (Sandoz) (fig.2). The effect was significant at a drug level of 7×10^{-11} M ($P < 0.01$).

The influence of dopamine and bromocryptine on TRH-stimulated and unstimulated prolactin release by the cultures was also determined in each experiment. The effects on prolactin secretion were found to parallel those on TSH secretion however prolactin secretion was inhibited by lower (~10-fold) concentrations of dopamine and bromocryptine (not shown).

The effect of dopamine on TSH secretion by TRH-stimulated cells could be inhibited in a dose-dependant manner by the dopamine antagonists metoclopramide (Berke) and domperidone (Janssen) (fig.3). The effect of metoclopramide was significant ($P < 0.01$) at 10^{-10}

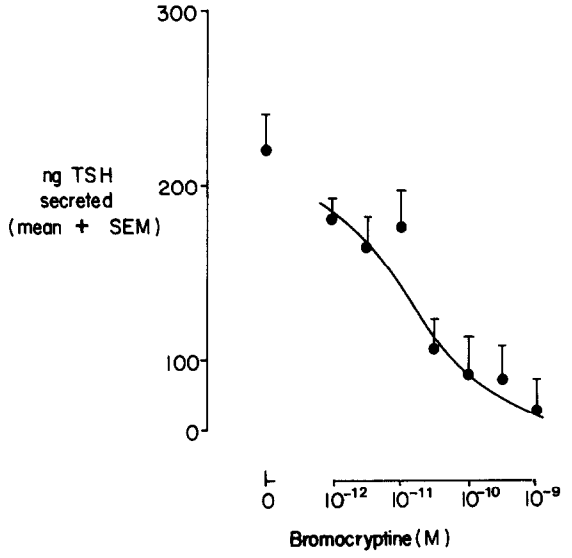

Fig.2. Inhibition of TSH secretion by the dopaminergic agonist bromocryptine.

M and domperidone at 10^{-8} M ($P < 0.01$). Metoclopramide and domperidone inhibited the effect of dopamine on TRH-stimulated prolactin secretion in a similar manner however the effects of these agents on dopamine inhibition of prolactin release were observed at an ~10-fold lower drug concentration (not shown).

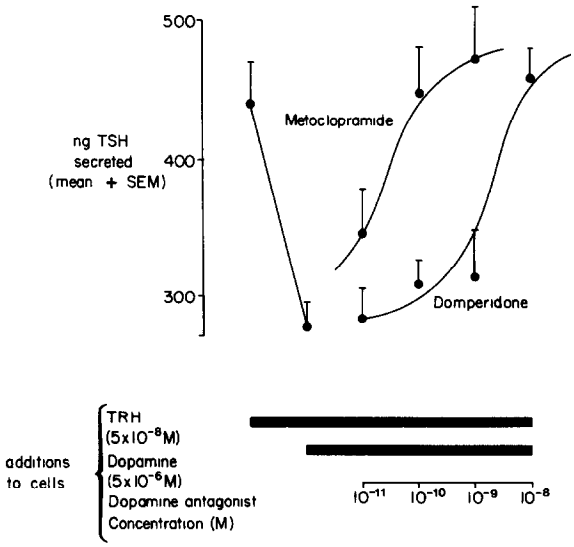

Fig.3. Effect of dopamine antagonists on dopaminergic inhibition of TSH secretion.

4. Discussion

These studies demonstrate that dopamine can inhibit TSH secretion by isolated pituitary cells. Furthermore the effects of dopamine can be mimicked by a dopamine agonist and inhibited by dopamine antagonists. This provides direct evidence that TSH secretion can be controlled by dopaminergic mechanisms at the level of the thyrotroph.

Although dopamine inhibited TSH secretion by stimulated and non-stimulated pituitary cells it did not have a significant effect on DBC-stimulated cells. The reason for this is unclear at present and this phenomenon clearly requires further investigation. Dopamine might act on events that occur earlier than cyclic AMP synthesis should this be the major modulator of TRH action [11,12].

Our studies of the effects of dopamine and prolactin secretion are in good agreement with studies on the effects of dopamine on prolactin secretion by isolated pituitary cells [9,13,14]. Likewise our studies are consistent with observations on the effects of dopamine agonists and antagonists on TSH and prolactin levels in man [1,15–17]. Consequently both in vitro and in vivo studies suggest that dopaminergic control of TSH secretion can occur at the level of the thyrotroph.

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