DOMPERIDONE DIRECTLY STIMULATES TSH SECRETION IN VITRO

Ruth POWELL, Michael C. WHITE, Mark DANIELS and Keith MASHITER
Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12 OHS, England

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1. Introduction

Administration of the dopamine antagonist, domperidone [1,2] stimulates TSH secretion in man [3]. Since dopamine suppresses both in vivo and in vitro basal and stimulated TSH secretion [4–8] and there is evidence that dopamine and domperidone do not cross the blood–brain barrier [1,2,9], this stimulatory effect of domperidone has been attributed to antagonism of dopaminergic inhibition of anterior pituitary TSH secretion [3]. As yet, however, a direct effect of domperidone on the thyrotroph has not been discounted.

Here, we demonstrate a direct stimulatory effect of domperidone on basal TSH secretion from rat anterior pituitary cells in culture, in the absence of dopamine. Dopamine alone did not modify basal TSH secretion, but did antagonise the TSH stimulatory effect of domperidone. In contrast, domperidone had no effect on basal prolactin secretion, but did antagonise dopaminergic inhibition of prolaction secretion.

These results suggest a differential sensitivity of lactotrophs and thyrotrophs to dopamine inhibition, and indicate that the in vivo TSH stimulatory effect of domperidone may not be entirely due to any dopaminergic antagonist properties of the drug.

2. Materials and methods

2.1. *Preparation of rat anterior pituitary cell cultures*

Female Wistar rats (180–220 g) were decapitated; the anterior pituitaries were removed and placed immediately in culture medium (Eagle's minimal essential medium containing 10% foetal calf serum (Gibco) 100 U/ml penicillin, 100 μg/ml streptomycin (Glaxo) and 2.5 μg/ml fungizone (Squibb), buffered with 20 mM Hepes (Hopkins and Williams) and 20 mM sodium bicarbonate.

The pooled anterior pituitaries were cut into ~1 mm^3 pieces, washed with phosphobuffered saline (pH 7.3) (Oxoid) and dispersed by 6 consecutive 20 min incubations at 37°C in 20 ml phosphobuffered saline containing 0.125% trypsin (Wellcome Reagents). Dispersed cells were aspirated and recovered by centrifugation (200 × *g*; 5 min). The resulting cells pellets were washed with 1 ml culture medium, pooled, recentrifuged and dispersed in 20 ml culture medium. Cells were counted using a haemocytometer and diluted with culture medium to give 2×10^5 cells/ml medium. Cell viability, determined by the trypan blue exclusion method, was >95%. 2.5×10^5 cells were added to glass tubes (Labco S100WC) and incubated at 37°C for 4 days to allow cell attachment prior to use in experiments.

2.2. *Experimental procedures*

Domperidone (Janssen Pharmaceuticals) was initially dissolved in 0.01 M HCl and subsequently diluted with ascorbate containing medium. The final maximum concentration of HCl used (5×10^{-6} M) did not modify prolactin or TSH secretion. Dopamine HCl (Arnar-Stone Labs) and thyrotrophin-releasing hormone (Roche) were diluted with ascorbate containing medium.

Prior to experiments, cells were washed with 2 ml culture medium. Experiments were performed at 37°C in closed tubes, using 2 ml culture medium containing 10^{-4} M ascorbic acid and appropriate drugs. A minimum of 5 cultures were used/treatment group. After a 4 h incubation, the culture medium was decanted and assayed for TSH and prolactin using reagents provided by Dr A. F. Parlow of the National Pituitary Agency. Prolactin secretion was expressed as ng NIAMDD-rat Prl-RP-2 and TSH as ng NIAMDD-rat TSH-RP-1. Data were analysed using the Student's '*t*'-test.

3. Results

Incubation of rat anterior pituitary cells with 2.4×10^{-9}–2.4×10^{-5} M domperidone for 4 h significantly ($p < 0.02$) increased TSH secretion. The same effect was observed in a second experiment (fig.1), in which 5×10^{-7} M dopamine decreased the stimulation produced by 2.4×10^{-9} M and 2.4×10^{-8} M ($p < 0.01$ and $p < 0.05$, respectively) but not 2.4×10^{-7} M domperidone. Similar results were obtained using a lower dose of dopamine (1×10^{-7} M). Dopamine had no effect on basal TSH secretion.

Domperidone had no effect on basal prolactin secretion at the concentrations tested (4.8×10^{-8} M and 2.4×10^{-7} M) but both doses blocked the inhibition produced by 5×10^{-7} M dopamine (fig.2). Thyrotrophin-releasing hormone (1.5×10^{-7} M) significantly stimulated TSH and prolactin release in all experiments ($p < 0.001$).

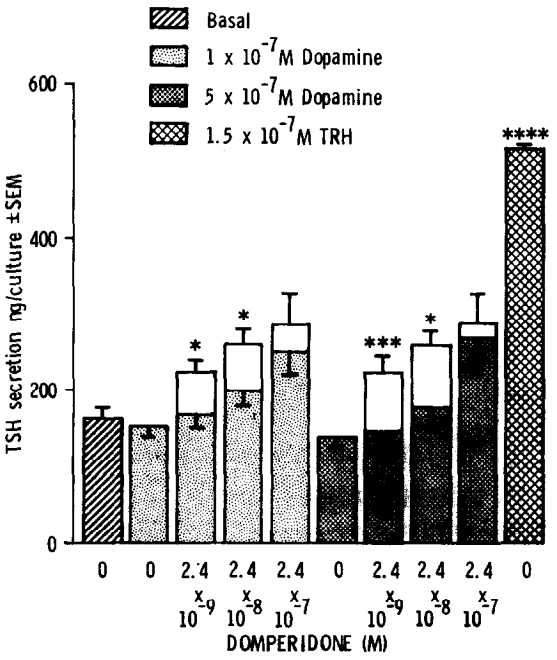

Fig.1. Effect of dopamine on basal and domperidone-stimulated TSH secretion from rat anterior pituitary cells in culture: $*p < 0.05$; $***p < 0.01$; domperidone *vs* domperidone- and dopamine-treated cultures.

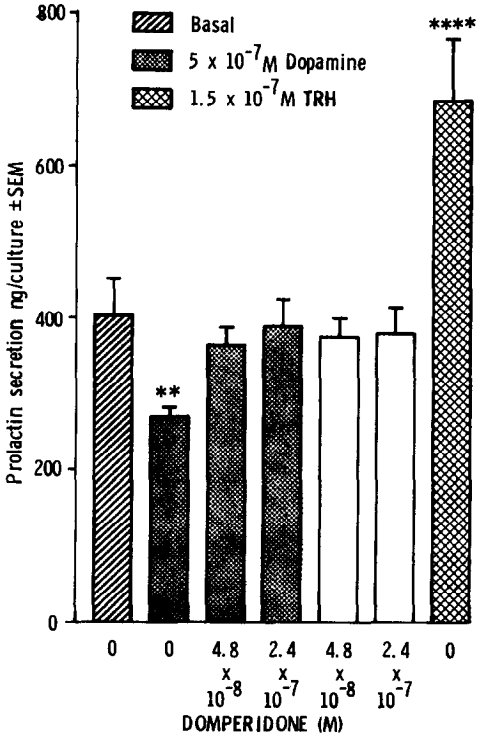

Fig.2. Effect of dopamine and domperidone on prolactin secretion from rat anterior pituitary cells in culture: $**p < 0.02$ *vs* ascorbic acid-treated cultures.

4. Discussion

Domperidone increases serum TSH and prolactin levels in man, and is believed to act by blocking the peripheral action of dopamine [3]. Our results, showing domperidone blocks the inhibitory effect of dopamine on prolactin secretion by rat anterior pituitary cells, are consistent with this view. However, the parallel demonstration that domperidone, when added alone, stimulates basal TSH secretion in vitro, suggests that this drug can act other than by dopamine blockade in respect of TSH secretion. The possibility that endogenous dopamine was present in the cultures would appear to be discounted, since domperidone had no effect on basal PRL secretion. Moreover, in these experiments we did not find an effect of dopamine on basal TSH secretion, although in vitro suppression has been reported [8]. Lack of dopamine suppression of TSH almost certainly relates to the use of lower concentrations in our studies. Nevertheless, these same doses significantly suppressed PRL secretion and are higher than levels reported to be present in rat hypophyseal portal blood [10–13]. Thus these results suggest a differential in the sensitivity of lactotrophs and thyrotrophs to dopamine.

The observed inhibition of domperidone-stimulated TSH secretion by dopamine may represent dopaminergic inhibition of stimulated TSH secretion, rather than any specific domperidone–dopamine interac-

tion at dopamine receptors. Inhibition of basal TSH secretion by dopamine was shown [8] using cells precultured for 24 h in dibutryl cyclic AMP, a stimulator of TSH release.

In conclusion, these results demonstrate that domperidone alone stimulates basal TSH secretion in vitro and suggest caution in attributing the in vivo TSH stimulatory effect of domperidone solely to dopamine antagonism. The results also indicate that dopamine would appear to be of lesser importance in the regulation of TSH than prolactin.

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