PHENOTHIAZINES INHIBIT PROLACTIN SECRETION IN VITRO

A possible role for calmodulin in stimulus–secretion coupling in the pituitary

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1. Introduction

Secretion of prolactin from the anterior pituitary gland is dependent on the presence of extracellular calcium. Manipulations which increase cytosolic calcium, such as incubation of pituitary cells with ionophores or with high potassium, stimulate hormone release [1–4]. Spontaneous depolarising action potentials have been recorded in both normal and tumour pituitary cells and, in general, thyrotropin-releasing hormone (TRH) increases and dopamine decreases these calcium-dependent action potentials [5–9]. In addition, agents which inhibit calcium flux also inhibit pituitary hormone secretion [1,4]. However, the precise role of intracellular calcium in the secretory process is unknown.

Many of the effects of calcium in tissues may be mediated through its interaction with the calcium-dependent regulator, calmodulin [10]. Calmodulin, a protein first discovered as a heat-stable activator of cyclic nucleotide phosphodiesterase [11], was later found to influence the activity of several other intracellular enzymes [12–14]. A number of neuroleptic drugs have been found to bind to calmodulin and to inhibit its action [15]. Among the most potent of these drugs are the phenothiazine tranquillizers [15]. In preliminary studies, we observed that trifluoperazine inhibited prolactin secretion from isolated pituitary cells [16]. Therefore, we have investigated the hypothesis that calmodulin has a role in prolactin secretion by studying the dose-dependence of the effects of two potent calmodulin inhibitors, trifluoperazine and prochlorperazine, on prolactin secretion in vitro. We have also investigated the effects of the sulphoxide derivatives of these drugs. Here, we report that both trifluoperazine and prochlorperazine inhibit prolactin secretion from rat anterior pituitary cells in vitro, but that the sulphoxide analogues have no such inhibitory effects.

2. Materials and methods

The culture medium (Ham's F10) was from Flow Labs (Irvine); the foetal calf serum, penicillin and streptomycin were from Gibco-Europe (Paisley). The trypsin was obtained from Difco Labs (West Molesey, Surrey) and the soya bean trypsin inhibitor was from Sigma (London) Chemical Co. (Poole, Dorset). Bovine serum albumin (fraction V) was from Armour Pharmaceuticals (Eastbourne, Sussex). TRH was a gift from Reckitt and Colman Ltd. (Hull) and the phenothiazines were gifts from Smith, Kline and French Labs (Welwyn Garden City, Herts). All other chemicals were from BDH Chemicals (Poole, Dorset).

Anterior pituitary cells were prepared from adult male Sprague-Dawley rats by sequential incubation in trypsin (0.25%; 45 min), then soya bean trypsin inhibitor (0.25%; 10 min) following which they were isolated by mechanical dispersion [17]. The cells were distributed in multiwell culture plates (70 000 cells/well) in Ham's F10 medium (with 10% foetal calf serum and 100 units/ml penicillin/streptomycin) and used in experiments 3 days later. The incubation medium was Krebs-Ringer bicarbonate (pH 7.6) with 0.5% bovine serum albumin and 0.2% glucose (KRB). After a 2 h preincubation in KRB, the cells were washed with KRB, then incubated in 1 ml KRB con-

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taining the secretagogues and drugs for 1 h at 37°C in an atmosphere of 95% O_2/5% CO_2. The test substances were made up in KRB with the exception of trifluoperazine sulphoxide, prochlorperazine and prochlorperazine sulphoxide which were solubilized first in dimethylsulphoxide (DMSO). The final concentration of DMSO in KRB was 1%. Trifluoperazine was also dissolved first in DMSO in those experiments comparing the effects of all the phenothiazines and equivalent concentrations of DMSO were added to the appropriate controls. When increased K^+ concentrations were used, isotonicity was maintained by a compensating reduction in the concentration of Na^+. Prolactin released into the medium was measured in triplicate by radioimmunoassay using materials supplied by the National Pituitary Agency, NIAMDD, National Institutes of Health (Bethesda MD). Separation of the bound and free moieties was achieved by precipitation of the bound fraction with donkey anti-rabbit antiserum. Inter- and intra-assay variations were <8.5%.

All the incubations were performed in triplicate within each experiment. The significances were assessed by analysis of variance.

3. Results

The effect of trifluoperazine on both basal and stimulated prolactin secretion in one experiment is shown in fig.1. The results of the separate experiments have been pooled and are shown in table 1. (Data are expressed as percentage change since basal release was variable.) It is clear that trifluoperazine exerts a dose-dependent inhibition on basal prolactin secretion at >4.2 μM. This phenothiazine inhibited prolactin secretion in the presence and in the absence of TRH. Prolactin secretion stimulated by increased extracellular K^+ concentration was also inhibited by trifluoperazine. The inhibitory effect appears to be less pronounced or even absent at 84 μM trifluoperazine. It is probable that the apparent lack of inhibition at 84 μM is due to cytotoxicity since a high proportion of the cells incubated with this dose (but not lower concentrations) of trifluoperazine took up trypan blue.

Table 1
Inhibition of prolactin secretion by trifluoperazine

Trifluoperazine (μM)	Basal (n = 6)	TRH (n = 4) (10 ng/ml)	K^+ (n = 4) (35 mM)
0	100	100	100
0.084	91.5 ± 6.4	94.1 ± 2.9	100.4 ± 8.1
0.42	94.4 ± 6.3	105.8 ± 7.6	97.9 ± 3.9
0.84	97.6 ± 9.0	99.6 ± 10.5	94.8 ± 5.1
4.2	83.9 ± 10.9	79.4 ± 8.5	87.4 ± 7.5
8.4	60.4 ± 7.2	58.7 ± 7.4	73.7 ± 4.6
42	52.3 ± 6.2	54.5 ± 8.3	56.2 ± 2.3
84	80.4 ± 11.5	74.6 ± 16.7	84.4 ± 21.1

Pooled data from separate experiments expressed as a percentage of the value in the absence of trifluoperazine (mean ± SEM)

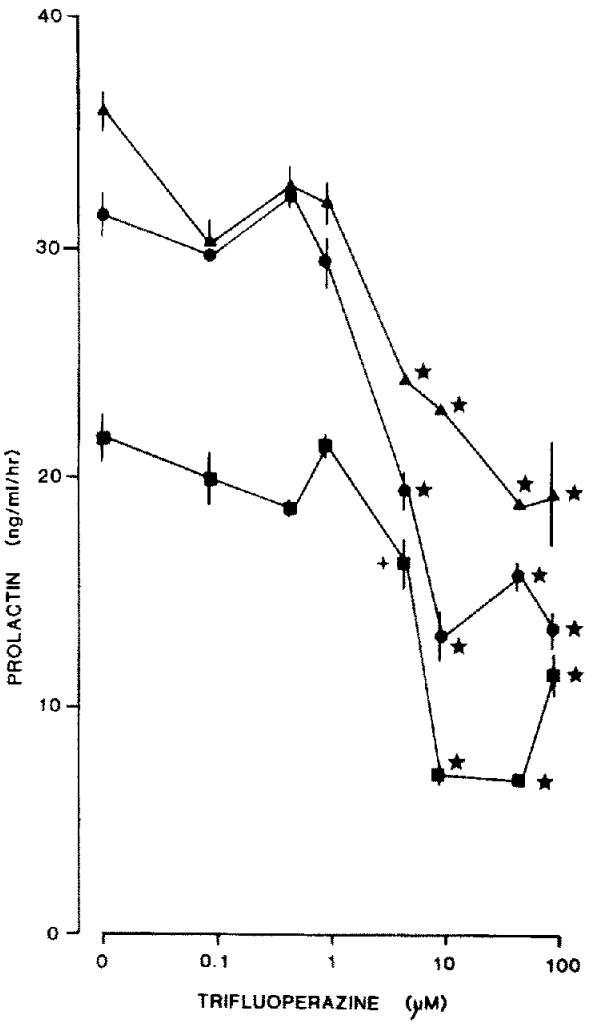

Fig.1. The effect of trifluoperazine on basal (■) TRH-stimulated (●) and K^+-stimulated (▲) prolactin secretion. $^+P < 0.02$, $^*P < 0.002$ for inhibition relative to the control levels of secretion. Prolactin secretion was stimulated by TRH and K^+ ($P < 0.002$).

Since the phenothiazine antipsychotics have been reported to have effects other than on calmodulin action, it was important to compare the effects of these phenothiazines with those of derivatives that are known (or suspected) to be less potent inhibitors of calmodulin action [15]. The results of these experiments are shown in fig.2, which depicts the effect of trifluoperazine, prochlorperazine and the sulphoxide derivatives on basal prolactin secretion. It is apparent that the sulphoxides were without inhibitory effect, in the dose range 0.084–84 μM, in the same experiments in which the parent compounds had profound inhibitory effects. Trifluoperazine sulphoxide is known to be considerably less potent as a calmodulin inhibitor [18,19]. The cause of the slight stimulatory effect of the prochlorperazine sulphoxide is not yet known.

4. Discussion

Trifluoperazine, a phenothiazine tranquillizer, inhibited prolactin release from rat anterior pituitary cells in vitro. In this study, the maximal effect was observed at 42 μM with an ED_{50} of ~6 μM (from 6 expt). This latter value is similar to the dose required for half-maximal effects of trifluoperazine on inhibition of calmodulin-activated phosphodiesterase [20] and on binding to calmodulin [15]. Trifluoperazine also inhibited prolactin secretion in the presence of TRH and of high K^+ concentration. Prochlorperazine,

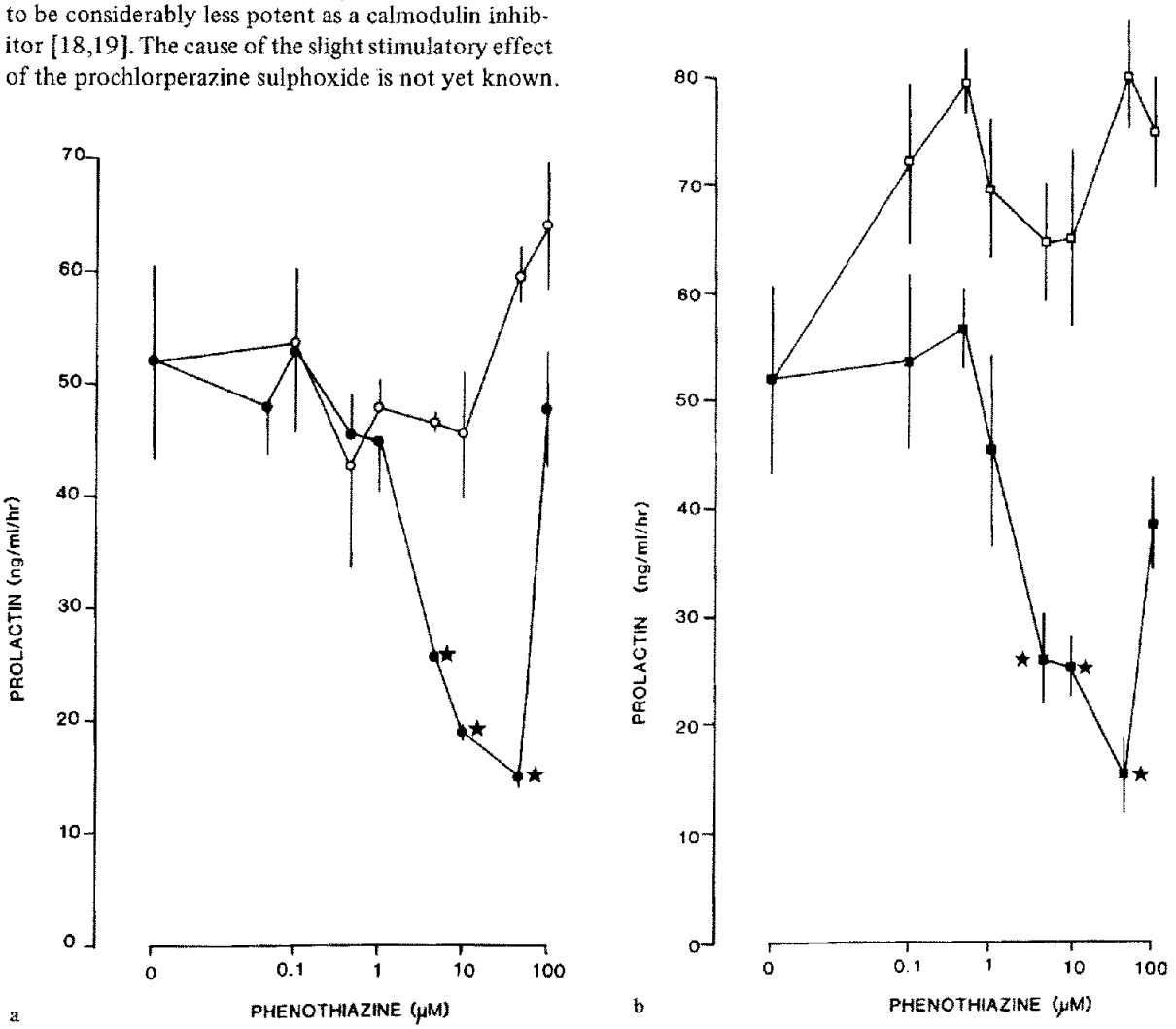

Fig.2. (a) The effect of trifluoperazine (•) and trifluoperazine sulphoxide (○) on basal prolactin secretion in a representative experiment. (b) The effect of prochlorperazine (▪) and prochlorperazine sulphoxide (□) on basal prolactin secretion in the same experiment. *$P < 0.01$ for inhibition relative to the control levels of secretion.

which has also been shown to bind to calmodulin and inhibit its action, was also effective in inhibiting prolactin secretion. The ED_{50} for this drug was ~5 μM, which again is similar to its known binding constant with calmodulin and the IC_{50} for the inhibition of calmodulin-activated phosphodiesterase [19]. These results suggest that the drugs may be interacting with calmodulin within the cell and that this interaction in some way leads to inhibition of the secretory process. However, these drugs may also have calmodulin-independent actions, such as a local anaesthetic-like action on Na^+ conductance [21] and interference with catecholamine binding to cells [22]. Nevertheless, the close relationship between the potencies of these drugs in inhibiting prolactin secretion and in affecting calmodulin action argues in favour of a calmodulin-dependent effect. This argument is strengthened by the finding that trifluoperazine sulphoxide did not influence hormone secretion. This derivative has been shown to have a lower (at least 1/30th) potency on inhibition of calmodulin-activated phosphodiesterase [18]. Moreover, the sulphoxide analogue of prochlorperazine, which is also less potent in inhibiting calmodulin-activated phosphodiesterase (unpublished) was also ineffective in inhibiting prolactin secretion.

Calmodulin may also be involved in insulin secretion [19,23,24], vasopressin-stimulated water flow [25], intestinal ion secretion [26,27] and stimulated thyrotropin secretion [28], in other cellular systems. However, this is the first report of a possible role for calmodulin in basal and stimulated prolactin secretion.

Since calmodulin activates cyclic nucleotide phosphodiesterase and calcium-dependent phosphorylation mechanisms it will be interesting to determine whether phenothiazines influence cyclic nucleotide (especially cyclic GMP) accumulation in pituitary cells and whether protein phosphorylation is affected. Such studies are now in progress in our laboratory.

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