

# Rapid inhibition by somatostatin of vasoactive intestinal peptide-induced prolactin secretion by rat pituitary cells

## Relationship to cyclic AMP accumulation

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*Isobutylmethylxanthine*

### 1. INTRODUCTION

Somatostatin (SRIF) has been shown to inhibit not only growth hormone secretion, but also the release of prolactin (PRL) both in vivo and in vitro [1–5]. On the other hand, vasoactive intestinal peptide (VIP) has been reported to stimulate specifically PRL release from rat pituitary lactotrophs [6]. It appears that this effect is mediated by cyclic AMP (cAMP) [7,8]. Here, we investigated whether SRIF can interact with the stimulatory effect of VIP on PRL release by rat pituitary cells in culture, and whether this action is mediated by a modification of the cAMP accumulation induced by VIP.

### 2. MATERIALS AND METHODS

#### 2.1. Cell culture

Male Wistar rats (45–60 days old) were used. In each experiment, 20 anterior pituitaries were dispersed enzymatically in minimal essential medium with Earle's salts containing 0.1% trypsin (1:250, Gibco) and 0.1% fatty-acid-free bovine serum albumin (BSA) for 2 h at 37°C at pH 7.5 in a silicone-treated Bellco spinner flask. After filtration to remove DNA and broken cells, and several washes,  $2\text{--}2.5 \times 10^5$  cells were plated in 16 mm tissue culture wells (Costar) in 250  $\mu$ l culture medium consisting in sterile medium 199 with Hank's salts (Gibco) containing 0.1% BSA, 25 mM Hepes, 10% horse serum, 2.5% fetal calf serum, and antibiotics, for 48 h at 37°C under 95% O<sub>2</sub>/5% CO<sub>2</sub>.

#### 2.2. Incubation

After 48 h, the culture medium was discarded and the cells were washed twice with filtered (0.45  $\mu$ m Millipore) Krebs Ringer (pH 7.5) phosphate buffer (118 mM NaCl; 5 mM KCl; 1.2 mM MgSO<sub>4</sub>; 1.2 mM KH<sub>2</sub>PO<sub>4</sub>; 10 mM Na<sub>2</sub>HPO<sub>4</sub>) containing 0.02 mM bacitracin (Sigma) and 2% BSA. All incubations were performed in 250  $\mu$ l of the same buffer at 25–26°C in the presence or absence of SRIF (synthetic SRIF, Beckman) for 15 min before the addition of highly purified porcine VIP (V. Mutt, Stockholm). The incubation was stopped 15 min after VIP addition. This time point was chosen since it allowed us to measure both cAMP accumulation and PRL release induced by VIP on the same cells [8].

#### 2.3. Assays

Aliquots of the medium (20–30  $\mu$ l) were taken for PRL measurement and the reaction was terminated by the addition of 25  $\mu$ l 1.1 M perchloric acid to the cells which were extracted for cAMP measurement by radioimmunoassay as in [8]. Medium PRL was measured by radioimmunoassay using reagents kindly supplied by the Pituitary Hormone Distribution Program (NIH) and Dr A.F. Parlow.

To represent the data from both cAMP and PRL by the same cells on the same figure, results were expressed as % of VIP stimulation. Means  $\pm$  SEM were obtained from the individual values and statistical analysis was performed by means of the non-parametric Friedman test [9].

## 3. RESULTS

As reported in [6,8], under similar experimental conditions, VIP induced a dose-dependent increase in cAMP accumulation and PRL release with a maximum increase occurring at  $10^{-7}$  M. Increasing [SRIF] from  $10^{-10}$ – $10^{-6}$  M inhibited in a dose-dependent manner the stimulation of both cAMP accumulation and PRL secretion induced by  $10^{-7}$  M VIP (fig.1). Half-maximal inhibition for both cAMP accumulation and PRL secretion was obtained with  $1-2 \times 10^{-10}$  M SRIF. However, if the cells are pretreated with 0.5 mM isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor, just before SRIF addition to the incubation medium, SRIF is able to dramatically inhibit PRL release induced by VIP without significantly counteracting the increase of cAMP accumulation induced by VIP (fig.2). Moreover, under these conditions, the dose-response curve of PRL secretion to VIP is attenuated by the presence of SRIF in the incubation medium 15 min before VIP (fig.3). SRIF at  $5 \times 10^{-7}$  M diminished the

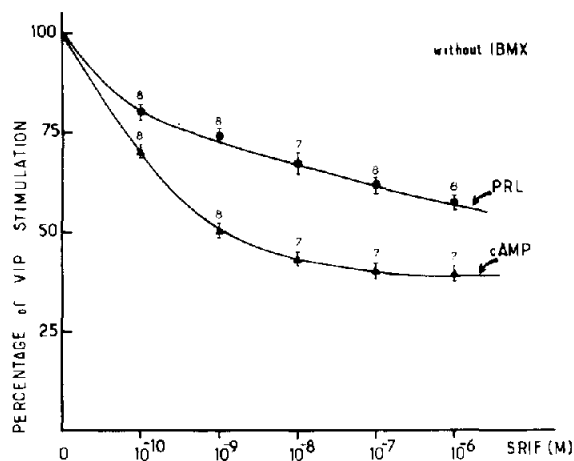


Fig.1. Effect of increasing [SRIF] on  $10^{-7}$  M VIP-induced stimulation of PRL release and cAMP accumulation (expressed as 100%) in the absence of IBMX. For VIP stimulation of both PRL and cAMP, 100% is equal to  $48 \pm 9$  ng/ $10^6$  cells and  $0.58 \pm 0.09$  pmol/ $10^6$  cells, respectively, which represent 3.4- and 2.3-fold increases as compared to basal values. Results are shown as means  $\pm$  SEM. Number of experimental points for each group are quoted.

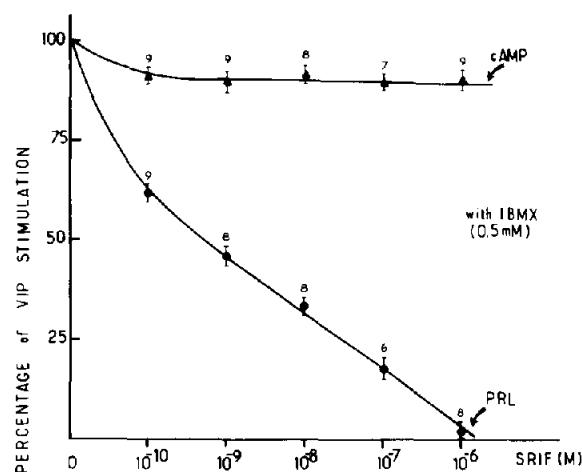


Fig.2. Effect of increasing [SRIF] on  $10^{-7}$  M VIP-induced stimulation of PRL release and cAMP accumulation (expressed as 100%) in the presence of 0.5 mM IBMX. For VIP stimulation of both PRL and cAMP, 100% is equal to  $68 \pm 10$  ng/ $10^6$  cells and  $1.16$  pmol/ $10^6$  cells, respectively, which represent 2.5- and 2.6-fold increases as compared to basal values. Results are shown as means  $\pm$  SEM. Number of experimental points for each group are quoted.

maximal stimulation of PRL release induced by VIP without significantly affecting the half-maximal stimulation (fig.3).

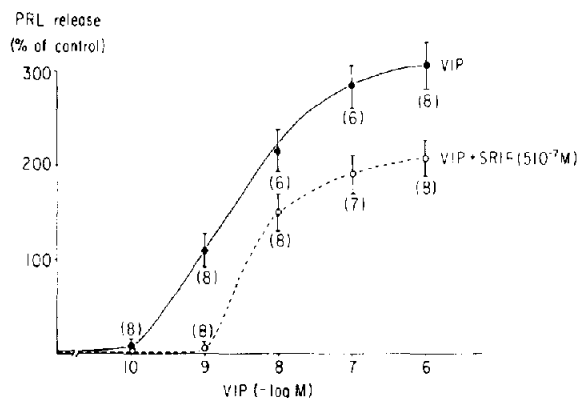


Fig.3. Dose-dependent stimulation of VIP-induced PRL release in the presence or absence of  $5 \times 10^{-7}$  M SRIF with IBMX. Results are shown as means  $\pm$  SEM. Number of experimental points are given in parentheses.

#### 4. DISCUSSION

These results show that VIP stimulation of PRL release by pituitary cells in culture can be modulated by SRIF. SRIF has been reported to inhibit the stimulation of PRL secretion by various neuropeptides such as TRH and bombesin [1,4,10] and by VIP on rat hemipituitaries [11]. The inhibitory effect of SRIF reported here on pituitary cells is dose-dependent and occurs rapidly since it can be obtained when SRIF is added to the incubation medium only 15 min before VIP. As reported for the inhibition of TRH-induced PRL release [1], the fact that SRIF blunts the maximal response of PRL release to VIP, without significantly affecting the apparent affinity of the effect, suggests that each peptide acts through separate receptor-binding sites. This conclusion can be related to the observation [12] that the specific binding of  $^{125}$ I-SRIF to pituitary membranes is not altered by VIP. This suggests that the possible interaction between SRIF and VIP takes place distally to the receptors.

In [7,8] VIP receptors in the pituitary were coupled to cAMP, and the increase of cAMP accumulation was related to stimulation of PRL secretion [7,8]. However, conflicting data have been reported regarding the involvement of cAMP in the mechanism of action of SRIF [13]. The correlation in the absence of a phosphodiesterase inhibitor between the inhibition by SRIF of cAMP accumulation and VIP-stimulated PRL release might be interpreted as indicating that the partial inhibitory effect of SRIF on VIP-induced PRL secretion is mediated by an inhibition of cAMP accumulation. However, the fact that, in the presence of IBMX, the inhibition by SRIF of PRL secretion induced by VIP is still observed and is even stronger, whereas cAMP is not significantly reduced, give little support to this hypothesis. These results with IBMX suggest that the effect of SRIF is not on the cAMP production, but indeed on a more distal step.

A possible alternative explanation however, for the dissociation observed in the presence of IBMX between cAMP accumulation on the one hand and PRL release induced by VIP on the other, is that SRIF may influence only a small portion of the intracellular cAMP pool, a portion which might be localized on the phosphorylation-dephosphoryla-

tion processes carried out by cAMP-dependent protein kinases [14]. Indeed, VIP was effective in stimulating cAMP-dependent protein kinases in isolated intestinal epithelial cells [15]. However, this effect could be difficult to detect when total cAMP is measured, as in these data, but still be efficient enough to affect PRL secretion. In the distal step, SRIF may have other actions: it could modify, for instance, the activity of the phosphodiesterase. However, if it would be the case, PRL secretion induced by VIP will not be affected by SRIF as for cAMP accumulation (fig.2). Finally, we cannot exclude the possibility that SRIF may act on the mobilization of intracellular calcium which may indirectly result in an impairment of the cyclic nucleotide metabolism [13]. Further work is now required to test these different possibilities.

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