SOMATOSTATIN INHIBITION OF RAT GROWTH HORMONE RELEASE IN VITRO IN THE PRESENCE OF $BaCl_2$ OR 3-ISOBUTYL-1-METHYLXANTHINE

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Received 11 July 1974

1. Introduction

Growth hormone release can be inhibited in vitro and in vivo by somatostatin, a tetra decapeptide isolated from ovine hypothalamus and characterized by Brazeau et al. [1]. This peptide may act on a secretory step occurring in several cell types, since it inhibits insulin and glucagon release in vivo [2]. In vitro the release of growth hormone can be stimulated by two types of secretogogue. The first type, which increases tissue cyclic AMP concentrations, includes phosphodiesterase inhibitors [3–5] and adenyl cyclase activators [6–8], and the second type includes the depolarizing agents high extracellular K^+ and Ba^{2+} which do not increase tissue cyclic AMP [5]. Since somatostatin has been shown to alter pituitary cyclic AMP concentrations [9], it might be expected to inhibit growth hormone release only in the presence of the first type of secretogogue. In this report we show that the apparent-K_i for somatostatin inhibition of Ba^{2+}-stimulated growth hormone release is the same as the apparent-K_i against 3-isobutyl-1-methylxanthine induced release, indicating that the same somatostatin-sensitive step is involved in the stimulation of growth hormone release by both reagents. This step probably does not involve cyclic AMP metabolism, since pituitary cyclic AMP contents were not changed on 10 min exposure to Ba^{2+} with or without added somatostatin, although Ba^{2+}-stimulated growth hormone release was inhibited by somatostatin over this time.

2. Materials and methods

2.1. *Tissue incubation procedure*

All incubations were performed in a bicarbonate buffered salt solution with the ionic composition: Na^+ 143.5 mM, K^+ 5.9 mM, Ca^{2+} 2.3 mM, Mg^{2+} 0.9 mM, Cl^- 129.4 mM, $H_2PO_4^-$ 1.2 mM and HCO_3^- 25 mM. The medium contained bovine serum albumin (1 mg/ml) and glucose (5.0 mM), and was equilibrated with O_2 : CO_2 (95:5). Pituitaries were taken from female rats (weight 110–140 g), and hemipituitaries weighing 1–2.5 mg and free of pars nervosa and pars intermedia were incubated in 0.5 ml medium with shaking, at 37°C. All pituitaries were preincubated for 90 min before the start of experiments. At the end of the incubation, the medium was removed and stored at −15°C for growth hormone assay, and the tissue was fixed for 90 min at 37°C in sodium phosphate (100 mM, pH 7.2) containing 2% glutaraldehyde and was then lightly blotted and weighed. Ultrastructural observations on the tissue will be presented elsewhere.

2.2. *Measurement of rat growth hormone*

Rat growth hormone reference standard (Rat GHRP-1), rat growth hormone for iodination (Rat GH-11-2) and anti-rat growth hormone serum (monkey, A-rat GHS-2) were obtained from The Hormone Distribution Officer, N.I.H. Bethesda. Growth hormone was iodinated using chloramine-T [10]. The growth hormone assay was performed in 200 μl barbitone (50 mM, pH 8.4) containing sodium azide (25 mM) and bovine serum albumin (1 mg/ml). Free labelled-growth hormone was absorbed onto charcoal in the presence of dextran and albumin [11], and removed by centrifugation: supernatant antibody-bound hormone was determined using a Packard 3002 γ counter.

2.3. *Tissue extraction and cyclic AMP assay*

When tissue cyclic AMP concentrations were measured, media were removed after a 10 min incubation, and 0.5 ml ice-cold TCA (5%) added to the tissue which was then homogenized. Protein was removed by centrifugation, redissolved in 0.5 N NaOH containing 5% sodium dodecyl sulphate and the concentration of protein determined using the Lowry procedure [12]. TCA supernatants were neutralized with Tris hydroxide (3.5 M), purified on Dowex 50WX8 [13] and freeze-dried in two aliquots. The lyophilate was reconstituted in 20 μl H_2O and the cyclic AMP concentration determined by a binding-protein assay [14].

3. Results

Growth hormone release was increased by 3-isobutyl-1-methylxanthine, the maximum stimulation being seen at 0.5 mM (fig. 1a). Addition of somatostatin (2 μg/ml) completely prevented this increase in release at all 3-isobutyl-1-methylxanthine concentrations tested. In the presence of 0.5 mM 3-isobutyl-1-methylxanthine, significant inhibition of release was observed in the presence of 2 ng/ml somatostatin, and half maximal inhibition at about 20 ng/ml (fig. 1b).

Growth hormone release was increased by $BaCl_2$ in the concentration range 0 to 8.8 mM, although this did not give maximum rates of release (fig. 2a). In the presence of 4.6 mM Ba^{2+}, growth hormone release was half maximally inhibited by 20 ng/ml somatostatin (fig. 2b). Addition of somatostatin at 2 μg/ml completely inhibited stimulation of growth hormone release by low concentrations of Ba^{2+}, but inhibition was partially overcome by increasing the $BaCl_2$ concentration to 8.8 mM (fig. 2a).

After 10 min incubation in the presence of 3-isobutyl-1-methylxanthine, pituitary cyclic AMP contents were increased 7.3 fold; 4.6 mM Ba^{2+} had no effect on cyclic AMP content (table 1). By contrast, growth hormone release was not significantly increased by 3-isobutyl-1-methylxanthine after 10 min, but was increased 3.6 fold by 4.6 mM Ba^{2+} and somatostatin inhibited this increase. Somatostatin decreased the

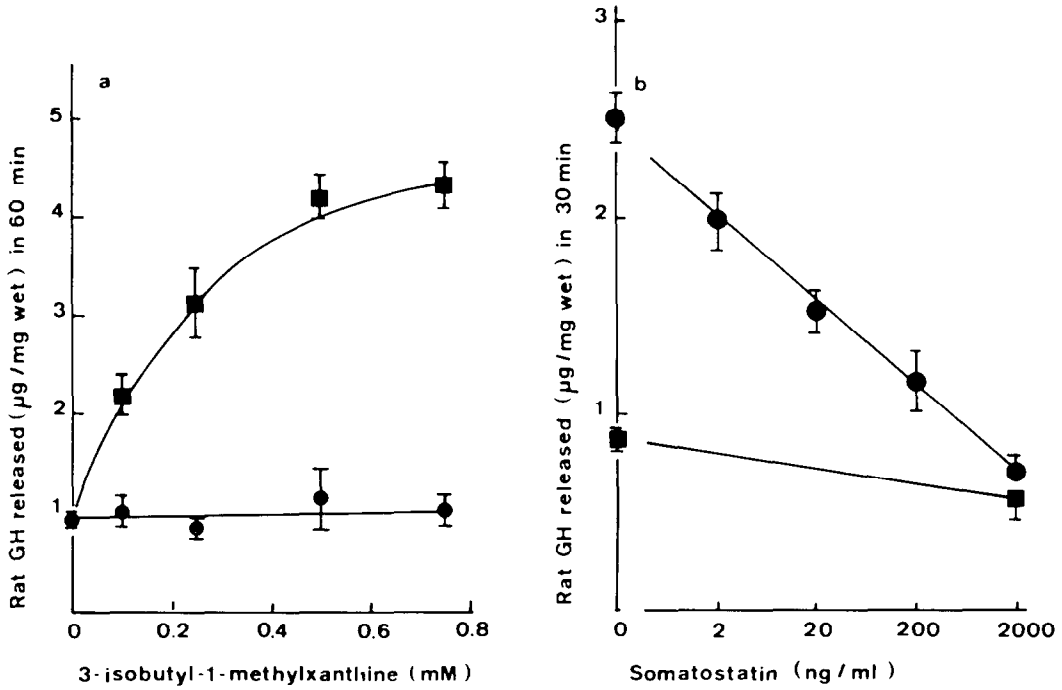

Fig. 1. Effect of somatostatin on 3-isobutyl-1-methylxanthine induced growth hormone release. Fig. 1a shows growth hormone release during 60 min in absence (■, n = 12) or presence (●, n = 4) of somatostain (2 μg/ml). Fig. 1b shows release during 30 min in absence (■, n = 8) or presence (●, n = 8) of 0.5 mM 3-isobutyl-1-methylxanthine. Vertical bars are standard error of mean.

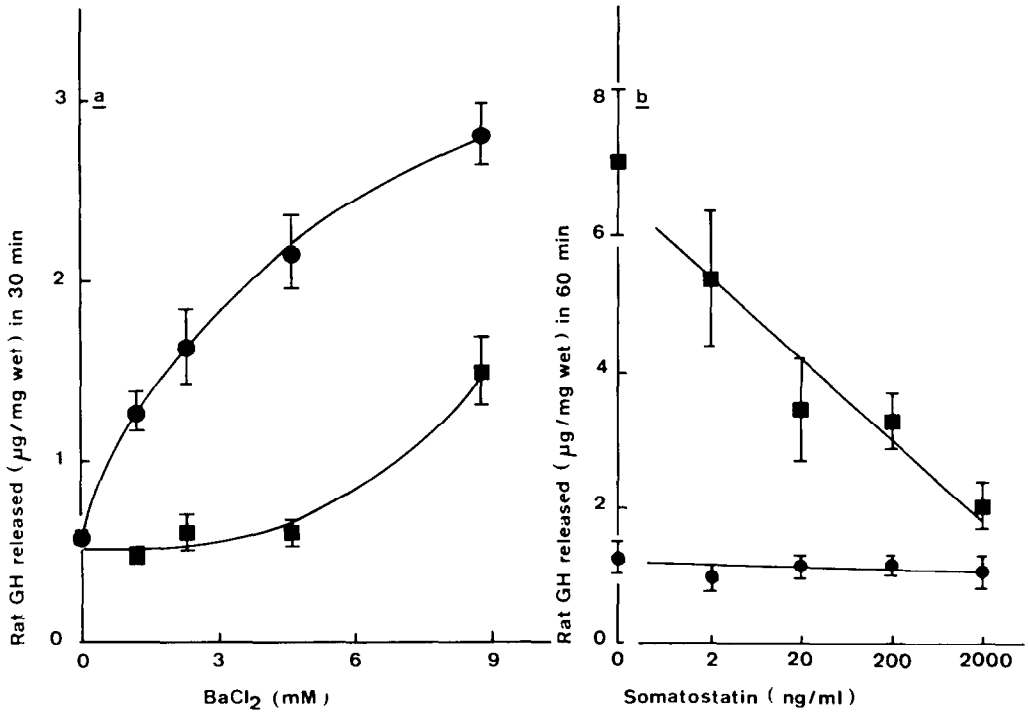

Fig. 2. Effect of somatostatin on Ba^{2+} induced growth hormone release. Fig. 2a shows growth hormone release during 30 min incubation in the absence (●, n = 8) or presence (■, n = 8) of somatostatin (2 μg/ml). Fig. 2b shows growth hormone release during 60 min in absence (■, n = 8) or presence (■, n = 8) of 4.6 mM $BaCl_2$. Vertical bars are standard error of mean.

Table 1
Effect of somatostatin on growth hormone release and cyclic AMP content of pituitaries incubated for 10 min

	GH release			Cyclic AMP content		
	(μg/mg protein) No somatostatin	(μg/mg protein) + somatostatin	(%)	(pmoles/mg protein) No somatostatin	(pmoles/mg protein) + somatostatin	(%)
Control	2.36±0.57	1.52±0.19	80±15	13.4± 0.46	12.0± 1.27	89±8
3-isobutyl-1-methylxanthine	3.07±0.27	2.17±0.39	76±15	98.0±15.0[a]	69.3±11.0	71±5[b]
Ba^{2+}	8.41±1.63[a]	3.11±0.50[a]	48±13[b]	11.0± 0.9	12.4± 0.6	115±6

Significance levels: a) $p < 0.01$ for difference from appropriate control without somatostatin; b) $p < 0.01$ for difference from 100%. Pituitaries were preincubated for 90 min and incubated for 10 min in 0.5 ml medium containing glucose (5 mM) and albumin (1 mg/ml). Concentrations of other reagents were: somatostatin 2 μg/ml; $BaCl_2$, 4.6 mM; 3-isobutyl-1-methylxanthine, 0.5 mM. Six half pituitaries were incubated without somatostatin and the 6 paired halves incubated under the same conditions but with somatostatin. Thus growth hormone release (or cyclic AMP content) in the presence of somatostatin is also expressed as % of the release (or cyclic AMP content) of the paired half pituitary incubated in the absence of somatostatin. Data is mean ± standard error.

pituitary cyclic AMP content by 30% in the presence of 3-isobutyl-1-methylxanthine; the difference became significant when expressed as % of paired control to eliminate interanimal differences in the number of somatotrophs. Somatostatin did not change cyclic AMP concentrations in control tissue, and may have slightly increased them in the presence of $BaCl_2$.

4. Discussion

These results show that in the rat hemipituitary as in the heifer pituitary slice [5], Ba^{2+} can increase growth hormone release without changing the intracellular concentration of cyclic AMP. Thus an increase in cyclic AMP is not essential for stimulation of either ox or rat growth hormone release. The rise in pituitary cyclic AMP appeared to precede the rise in hormone release, perhaps because it is indirectly linked to the secretory pathway. Thus $BaCl_2$ increased hormone release during a 10 min incubation, whereas 10 min exposure to 3-isobutyl-1-methylxanthine did not significantly increase growth hormone release despite increased cyclic AMP concentrations. A similar delay has been observed following prostaglandin E_2 [8].

The observation that the apparent-K_i for somatostatin inhibition of release stimulated by both secretogogues was 20 nm suggests that somatostatin affects the same secretory step in the presence of both stimuli. Somatostatin could inhibit this step by decreasing the concentration of cyclic AMP, since this might inhibit Ba^{2+}-induced growth hormone release : the converse effect, increased Ba^{2+}-induced release from pituitary slices containing increased cyclic AMP concentrations, has been reported [5]. The data presented here do not support this explanation. Somatostatin did not significantly decrease cyclic AMP concentrations in control pituitaries and may even have increased them in the presence of $BaCl_2$. An alternative explanation would be that somatostatin alters the distribution of Ca^{2+} in the tissue or its interaction with the secretory pathway. Our observation that increasing the $BaCl_2$ concentration in the presence of somatostatin was able to stimulate growth hormone release could suggest some competition between the peptide and divalent cation.

It is therefore of interest that somatostatin decreased pituitary cyclic AMP content in the presence of 3-isobutyl-1-methylxanthine. In the female pituitaries used in these studies, 33 ± 2.5% of all cells are somatotrophs (based on 2186 cells in sections from 12 pituitaries). Thus a fall of 29 ± 4.6% in pituitary cyclic AMP content could represent virtually complete inhibition of cyclic AMP accumulation in somatotrophs, either through inhibition of cyclic AMP production or through reversal of 3-isobutyl-1-methylxanthine action. It is not known how far a change in intracellular cyclic AMP concentration could be explained by the postulated effect of somatostatin on divalent cation metabolism.

Acknowledgements

We are grateful to Dr R. Guillemin for his generous gift of linear somatostatin, and to Dr W. Malaisse for interest and advice. The work was supported by grant No 3.8080.72 from the Fonds National Suisse de la Recherche Scientifique.

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