MECHANISM OF ACTION OF SOMATOSTATIN: INHIBITION OF IONOPHORE A23187-INDUCED RELEASE OF GROWTH HORMONE FROM DISPERSED BOVINE PITUITARY CELLS

R. J. BICKNELL and J. G. SCHOFIELD
Department of Biochemistry, University of Bristol, Bristol, BS8 1TD, England

Received 5 July 1976

1. Introduction

Growth hormone secretion can be inhibited by a peptide, somatostatin, present in the hypothalamns and median eminence [1]. The mechanism of this inhibition is not known, but since somatostatin inhibits the secretion of several hormones in response to a wide range of in vitro stimuli [1–5] it probably affects a process common to the secretory pathways in all these cell types. This process may involve calcium ions since, for many cell types, a role for calcium in stimulus-secretion coupling has been suggested by several experimental techniques [6]. Some data show that the inhibition of hormone secretion by somatostatin can be reversed by increasing the concentration of calcium [3,7] or barium [8] ions, which suggest that somatostatin could affect the provision of calcium to a secretory step.

Recently, the antibiotic ionophore A23187, which can complex with calcium and facilitate its movement through biological membranes [9] has been shown to stimulate secretion, increasing for example the release of histamine from mast cells [10]. If inhibition of growth hormone secretion by somatostatin does indeed involve a decrease in the permeability of the cell membrane to calcium, A23187 would be expected to reverse the inhibition by overcoming the membrane permeability barrier. However, A23187 did not increase growth hormone release from rat hemipituitaries, perhaps because of its poor penetration in the tissue [11]. We have therefore investigated the effect of A23187 on growth hormone secretion from dispersed cells isolated from bovine anterior pituitaries by an enzymatic procedure, and the sensitivity of this secretion to somatostatin.

2. Materials and methods

2.1. *Cell dispersion*

A pituitary gland removed from a heifer within 5 min of slaughter was sliced [12] and incubated at 37°C for 15 min in incubation medium A (composition: NaCl, 118 mM; KCl, 5.9 mM; $CaCl_2$, 0.25 mM; $MgCl_2$, 0.3 mM; $NaHCO_3$, 25 mM; and $KHPO_4$, 1.2 mM; glucose, 2.8 mM; sodium 3-hydroxybutyrate, 1.2 mM; penicillin (50 μg/ml); streptomycin, 40 μg/ml; bovine serum albumin (Sigma, fraction V) 2 mg/ml; containing the M.E.M. amino acid supplement and equilibrated with O_2:CO_2 (95:5)). The slices were then cut into fragments 1 mm square and stirred for 10 min in 10 ml medium A containing 10 mg Trypsin (Sigma Type III). The tissue fragments were then recovered by centrifugation, washed with 10 ml medium A containing 7.5 mg Soyabean Trypsin Inhibitor (Sigma Type I-S), and then incubated with stirring in 10 ml medium A containing Soyabean Trypsin Inhibitor and 960 units of collagenase (Sigma Type II). After 45 min the tissue fragments were recovered by centrifugation and partially dispersed in 10 ml medium A by drawing them up about 40 times into a 5 ml plastic syringe through a plastic tube (5 × 0.25 cm). Undigested tissue fragments were allowed to settle for 2 min, the supernatant containing dispersed cells was removed and the tissue fragments stirred for 15 min in the original collagenase

containing medium. At the end of this incubation the dispersion procedure was repeated and the two cell suspensions pooled. The suspension was then filtered through a nylon gauze (68 GGN: Henry Simon Ltd, Stockport, Cheshire, SK3 0RT). A yield of approximately 25 X 10^6 cells was obtained, and the cells were 85–90% viable by Trypan Blue exclusion.

2.2. *Cell perifusion*

The cells were removed by centrifugation (Position 1 on MSE bench centrifuge) and approximately 12 X 10^6 cells incubated for 120 min in 1 ml medium B (Composition:NaCl, 118 mM; KCl, 5.9 mM; $NaHCO_3$, 25 mM; KH_2PO_4 1.2 mM; glucose, 2.8 mM; sodium 3-hydroxybutyrate 1.2 mM; albumin 0.5 mg/ml) containing $^{45}Ca^{2+}$ (20 μC_1; 23 μM). The cells were then recovered by centrifugation, resuspended in 1 ml fresh medium B and 300 μl aliquots added to three PVC columns (0.4 X 0.75 cm) containing Sephadex G-10 acetylated to decrease $^{45}Ca^{2+}$ adhesion [13]. The columns were then placed in a 37°C cabinet and eluted with medium B containing 2.5 mM $CaCl_2$, at a flow rate of 0.2 ml/min: collecting 2 min fractions. At the end of this perifusion, media were stored at −20% before radioimmunoassay of growth hormone [12] and measurement of $^{45}Ca^{2+}$ by liquid scintillation.

Linear somatostatin was given by Dr R. Guillemin of the Salk Institute, and was stored at −20°C as a 1 mg/ml solution in 0.9% NaCl. A23187 was obtained from Dr Otto Behrens of Eli Lilly, and was stored at −20°C as a 10 mg/ml solution in dimethylsulphoxide (DMSO).

3. Results

Release of growth hormone from bovine pituitary slices [14] and from rat hemipituitaries [8] is stimulated by $BaCl_2$, and this stimulation is sensitive to somatostatin inhibition [8]. Release of growth hormone from the dispersed bovine pituitary cell preparation was also stimulated by $BaCl_2$, and inhibited by somatostatin. The data in fig.1 represent the average for three experiments on three cell preparations and show that during perifusion with $BaCl_2$ (2 mM) in the presence of somatostatin (1 μg/ml) considerably less growth hormone was released than during a subsequent perifusion with $BaCl_2$ alone. After

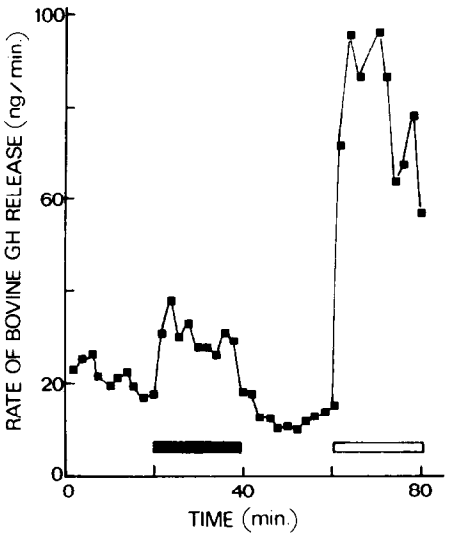

Fig.1. Effect of $BaCl_2$ and somatostatin on bovine GH release from perifused pituitary cell preparations. Data show mean bovine GH release obtained from perifusion of three pituitary cell preparations with medium in which $BaCl_2$ (2 mM) was present during the period shown by the open bar and in which $BaCl_2$ (2 mM) and somatostatin (1 μg/ml) were present during the period shown by the solid bar.

removal of $BaCl_2$ and somatostatin, the rate of growth hormone release returned rapidly to basal values.

A23187 (10 μg/ml) also increased growth hormone release from the dispersed cells; in four experiments, the mean rate of release during 20 min in the presence of A23187 was 0.866 ± 0.013 μg/min compared to 0.216 ± 0.013 μg/min in control medium during the previous 20 min. Addition of DMSO alone did not alter growth hormone release (data not shown). The increase in growth hormone release caused by A23187 was inhibited by somatostatin (1 μg/ml); with the same four preparations the mean rate of release during 20 min perifusion in the presence of A23187 and somatostatin was 0.386 ± 0.028 μg/min compared to 0.220 ± 0.009 μg/min in the previous 20 min. Neither A23187 nor somatostatin affected the growth hormone assay (data not shown). Following removal of A23187 from the medium, the rate of growth hormone release slowly decreased towards basal values (fig.2a); it is not clear whether this slow reversal represents slow removal of A23187 from cell membranes or a slow restoration of the normal internal ion concentrations.

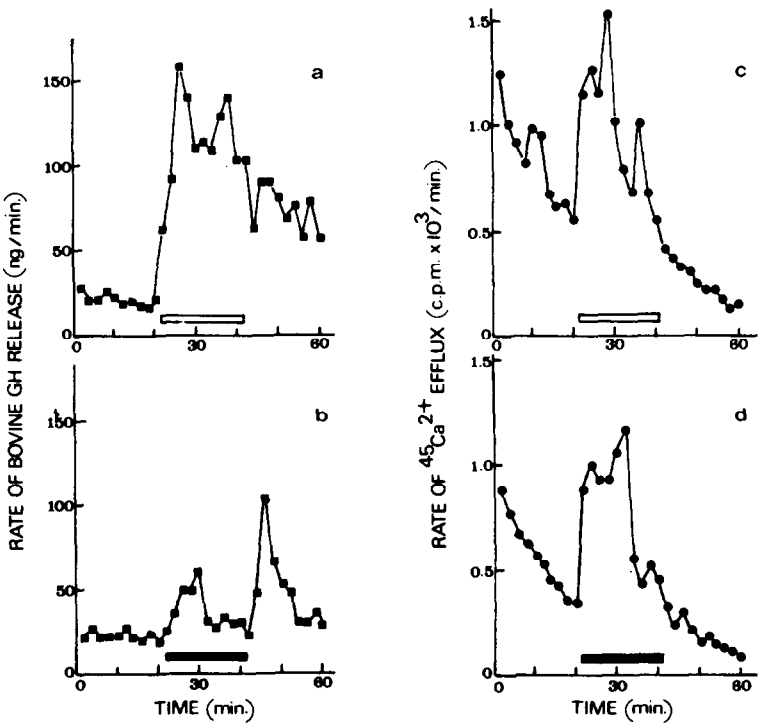

Fig.2. Effect of A23187 and somatostatin on bovine GH release and $^{45}Ca^{2+}$ efflux from perifused pituitary cell preparations. Figs. 2a and 2c show the mean bovine GH release and $^{45}Ca^{2+}$ efflux from two pituitary cell preparations perifused with medium in which A23187 (10 μg/ml) was present during the period shown by the open bar. Figs.2b and 2d show the corresponding data obtained from identical aliquots of the same two pituitary cell preparations perifused in parallel with medium in which A23187 (10 μg/ml) and somatostatin (1 μg/ml) were present during the period shown by the solid bar.

However, following removal of A23187 and somatostatin there was a transient increase in growth hormone release as would be expected if the effect of somatostatin was more rapidly reveratsed than that of A23187 (fig.2b).

As would be expected if membrane calcium permeability were altered, A23187 increased the rate of $^{45}Ca^{2+}$ efflux from the cells (fig.2c); since the external calcium concentration was 2.5 mM, this efflux of radioactivity presumably represents exchange rather than net calcium movement. Somatostatin did not prevent the increase in $^{45}Ca^{2+}$ efflux (fig.2d).

4. Discussion

The experiments reported here show that A23187 can increase growth hormone release from dispersed bovine pituitary cells. The lack of effect of the ionophore on growth hormone release from intact tissue previously reported [11] was therefore presumably due to poor penetration of the tissue by the ionophore. The ability of somatostatin to inhibit the growth hormone release induced by A23187 strongly suggests that the release did not result from cell damage, and is of some interest to understanding the mechanism of somatostatin action. It has been suggested that somatostatin acts by decreasing tissue cyclic AMP [15] or increasing cyclic GMP [16] concentrations, by inhibiting calcium entry into cells [5] or inhibiting a calcium-dependent process in secretion [7]. The data presented here show that although somatostatin inhibits the growth hormone release from pituitary cells induced by A23187, it does not prevent the $^{45}Ca^{2+}$ efflux caused by the ionophore. The detailed explanation of this $^{45}Ca^{2+}$ efflux is

complex, but since the external Ca^{2+} concentration was 2.5 mM, it presumably represents an increase in isotope exchange from tissue stores following an increase in cytoplasmic calcium concentration caused by the ionophore. Since somatostatin did not affect this efflux, it does not apparently modify the action of A23187 on membrane calcium permeability or the redistribution of calcium. Thus it would appear that somatostatin can inhibit the secretory process in the presence of calcium at stimulatory concentrations in the anterior pituitary. It is interesting to contrast these results with those obtained in isolated pancreatic islets where somatostatin (1 μg/ml) potentiated insulin and glucagon release in the presence of A23187, which was explained as being due to decreased calcium efflux [5].

Acknowledgements

This work was supported by a grant from the M.R.C. The authors also wish to thank Dr Roger Guillemin for the gift of somatostatin and Miss Hilary Cross for technical assistance.

References

[1] Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J. and Guillemin, R. (1973) Science 179, 77–79.
[2] Hayes, J. R., Johnson, D. G., Koerker, D. and Williams, R. H. (1975).
[3] Okamoto, H., Noto, Y., Miyamoto, S., Mabuchi, H. and Takeda, R. (1975) FEBS Lett. 54, 103–107.
[4] Fujimoto, W. Y. (1975) Endocrinology 97, 1494–1502.
[5] Fujimoto, W. Y. and Ensink, J. W. (1976) Endocrinology 98, 259–261.
[6] Rubin, R. P. (1970) Pharmacol. Rev. 22, 389–428.
[7] Curry, D. L. and Bennett, L. L. (1976) Proc. Natl. Acad. Sci. USA 73, 248–251.
[8] Schofield, J. G., Mira-Moser, F., Schorderet, M. and Orci, L. (1974) FEBS Lett. 46, 171–174.
[9] Scarpa, A. and Inesi, G. (1972) FEBS Lett. 22, 275–276.
[10] Foreman, J. C., Mongar, J. L. and Gomperts, B. D. (1973) Nature 245, 249–251.
[11] Mira-Moser, F., Schofield, J. G. and Orci, L. (1976) Europ. J. Biochim. Invest. 6, 103–111.
[12] Schofield, J. G. (1967) Biochem. J. 103, 331–341.
[13] Reed, K. C. (1973) Biochem. Biophys. Res. Comm. 50, 1136–1142.
[14] Schofield, J. G. and McPherson, M. (1974) Biochem. J. 142, 295–300.
[15] Borgeat, P., Labrie, F., Drovin, J., Belanger, A., Immer, H., Sestanji, K., Nelson, V., Gotz, M. and Schally, A. V. (1974) Biochem. Biophys. Res. Comm. 56, 1052–1059.
[16] Kaneko, T., Oka, H., Munemura, M., Suzuki, S., Yashida, H., Oda, T. and Yanaihara, N. (1974) Biochem. Biophys. Res. Comm. 61, 53–57.