RELEASE OF GROWTH HORMONE FROM OX PITUITARY SLICES AFTER PRONASE TREATMENT

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Proteolytic enzymes have been used both to modify properties of the cell membrane and to dissociate cells from many tissues including the pituitary (4, 5, 12). Exposure of secretory tissues to pronase can alter their secretory response. Thus incubation of pancreatic islets of Langerhans in the presence of low concentrations of pronase increased the subsequent release of insulin in the presence of stimulatory and nonstimulatory glucose concentrations (7). The purpose of the present investigation was to determine whether low concentrations of pronase have the same stimulatory effect on the release of a pituitary hormone, growth hormone. Such an effect on hormone release could be of some importance in view of the development of dissociated cell systems as models for the study of the control of hormone release (4, 5).

MATERIALS AND METHODS

Pituitary slices, prepared from pituitary glands removed from heifers within 5 min of slaughter as described elsewhere (8), were incubated at 37°C in Krebs-Henseleit bicarbonate-buffered salt solution containing glucose (5.5 mM) and sodium DLβ hydroxybutyrate (2.5 mM) during transport to the laboratory. The slices were then transferred to 10-ml flasks and incubated at 37°C, with shaking, for 90 min in 2 ml medium with or without pronase (4 μg/ml, Sigma protease repurified type VI, Sigma Chemical Co., St. Louis, Mo.). At the end of this preincubation the medium was removed and the slices were thoroughly washed by four successive additions of 2 ml medium to remove pronase from the tissue and the flask. The slices were then incubated at 37°C for 45 min in 2 ml medium modified as described in legends to the figures. All media were equilibrated with O2-CO2 (95:5) before and during incubation. At the end of the incubation the medium was removed for measurement of growth hormone concentration by double antibody radiomunoassay (8), and the tissue was fixed at room temperature in 2% glutaraldehyde in phosphate buffer (100 mM, pH 7.2) and processed for electron microscopy.

RESULTS

Stimulation of Growth Hormone Release by Pronase

Fig. 1 shows that pronase treatment approximately doubled basal release of growth hormone (from $0.93 \pm 0.16$ to $2.23 \pm 0.23 \mu g/mg$ wet wt, $n = 24$) and also doubled release in the presence of prostaglandin $E_2$ (from $4.14 \pm 0.59$ to $8.19 \pm 0.77 \mu g/mg$ wet wt, $n = 8$). Release in the presence of 71 mM K+, at which concentration K+ exerts its maximum effect (9), was increased by 60% (from $7.71 \pm 0.44$ to $12.09 \pm 0.78 \mu g/mg$ wet wt, $n = 24$). Thus the increase in growth hormone release resulting from pronase treatment was not constant, as would be expected if a few somatotrophs were damaged by the enzyme and released all their hormone during subsequent incubation. Instead, this increase in secretory response was greater in stimulated than in unstimulated tissue, suggesting that pronase in some way modified the secretory response.

The reversibility of the pronase effect was investigated in two ways. Pituitary slices exposed for 90 min to pronase were washed, incubated for 45 min in medium containing 71 mM K+, and then for three further 45-min periods in normal medium (Fig. 2 b). The release of growth hormone during these last three incubations was identical whether or not the tissue had been treated with pronase, indicating that this treatment did not alter the reversibility of K+ stimulation. Moreover, the secretory response of slices exposed to 71 mM K+ was not significantly greater 135 min after pronase pretreatment than the response of untreated slices...
FIGURE 1 Effect of pronase pretreatment on growth hormone release. The data show the growth hormone output of pituitary slices incubated for 60 min in control medium, in the presence of prostaglandin (1 μM), 71 mM K+ medium (in which the Na+ content was reduced to 77 mM), or 71 mM K+ medium containing NiCl₂ (1.0 mM). The bars show SEM, and shaded columns represent slices pretreated for 90 min with pronase (4 μg/ml).

(Fig. 2a) (10.75 ± 1.35 cf. 8.27 ± 1.02 μg/mg wet wt, n = 16), suggesting that the effect of pronase is only temporary.

Evidence that the effect of pronase is not due to cell lysis was provided by the inhibition of the response by NiCl₂ (1 mM). At this concentration, NiCl₂ inhibits release of insulin and amylase as well as growth hormone in response to several stimuli (3). Fig. 1 shows that NiCl₂ (1 mM) decreased growth hormone release in the presence of 71 mM K+ to the same rate whether or not the tissue had been incubated in pronase (1.76 ± 0.16 for pronase-treated and 1.95 ± 0.11 μg/mg wet wt for untreated tissue).

Effect of Pronase on Pituitary Morphology

Pronase treatment did not appear to alter the general morphology of the pituitary, in the sense that the frequency of occurrence of lysed or damaged cells was not apparently increased; a low magnification of pronase-treated tissue is shown in Fig. 3. A major feature of incubated heifer pituitary tissue was the extensive fusion of secretory granules to form exocytotic channels. These channels were occasionally seen in control tissue, and pronase-treated tissue incubated in the absence of stimuli (Fig. 4a), but they were most frequently encountered in tissue exposed to high-K+ either with or without pronase pretreatment (Fig. 4b, c). The number of cells containing these channels varied in different regions of the tissue, and the extent of the channels, together with the fact that the granule contents were dissolving in them, also prevented a quantitative determination of the number of granules liberated by exocytosis in each condition. The existence of such extensive invaginations of what is, effectively, the cell membrane is of some interest, since it suggests that in stimulated cells the secretory granules do not have to move large distances to approach the cell membrane.

DISCUSSION

The results presented here show clearly that exposure of pituitary tissue to pronase at concentrations three orders of magnitude below those needed to dissociate the tissue (5) increases the subsequent release of growth hormone. One obvious mechanism for this increase could be passive release of hormone from cells damaged by the enzyme. The quantitative data do not support this suggestion, however; pronase pretreatment increased basal release by 1.30 μg/mg wet wt but increased prostaglandin-induced release by 4.03 μg/mg wet wt under conditions when the same number of damaged cells should have been present. Moreover NiCl₂ inhibited hormone release from pronase-treated slices as effectively as from untreated slices. Qualitatively, no effect on the preservation of the tissue was observed. Therefore,
FIGURE 3  Bovine anterior pituitary incubated for 90 min in pronase (4 μg/ml) and then for 60 min in control medium. The electron micrograph shows the satisfactory degree of preservation of several secretory cells. × 5,000.
Fig. 4 shows exocytosis (arrows) in pronase-pretreated tissue incubated for 60 min in control medium. Fig. 4 b shows exocytosis in pituitary tissue incubated for 60 min in 71 mM K⁺ without pronase pretreatment; and Fig. 4 c shows exocytosis in pituitaries incubated for 60 min in 71 mM K⁺ after pronase pretreatment. Extensive dissolution of granule contents is seen in Fig. 4 c. Fig. 4 a, × 17,000. Fig. 4 b, × 20,000. Fig. 4 c, × 20,000.
it is possible that pronase enhances the ability of the pituitary to respond to secretory stimuli.

The mechanism of this enhancement is not clear, and the biochemistry of the events which intervene between the addition of prostaglandin E₂ or increase in the K⁺ concentration and the release of growth hormone is equally not known. Prostaglandin E₂ may act by increasing the concentration of cyclic AMP in the pituitary (2), and high K⁺ leads to depolarization (6) and increased Ca²⁺ incorporation (11) without changing the cyclic AMP concentration (10). Since pronase potentiates the effect of both stimuli it is probable that it affects a fundamental property of the secretory system, for example, membrane Ca²⁺ permeability.

Alternatively, since the release of growth hormone is known to be inhibited by the hypothalamic peptide somatostatin (1), pronase could increase release by destruction of somatostatin present in the tissue at the time it was removed from the animal.

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REFERENCES

1. BRAZEAU, P., W. VALE, R. BURGUS, N. LING, M. BUTCHER, J. RIVIER, and R. GUILLEMIN. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science (Wash. D.C.). 179:77–79.

2. COOPER, R. H., M. MCPHERSON, and J. G. SCHOFIELD. 1972. Effect of prostaglandins on ox pituitary content of adenosine 3':5'-cyclic monophosphate and the release of growth hormone. Biochem. J. 127:143–154.

3. DORMER, R. A., A. L. KERBEY, M. MCPHERSON, S. MANLEY, S. J. H. ASCROFT, J. G. SCHOFIELD, and P. J. RANDLE. 1974. Effect of nickel on secretory systems; studies on the release of amylase insulin and growth hormone. Biochem. J. 140:135–142.

4. HOPKINS, C. R., and M. G. FARGHER. 1973. Hormone secretion by cells dissociated from rat anterior pituitaries. J. Cell Biol. 59:276–303.

5. LLOYD, R. V., and W. H. MCINNISH. 1973. Study of rat anterior pituitary cells separated by velocity sedimentation under unit gravity. Endocrinology. 92:1639–1651.

6. MILLIGAN, J. V., and J. KRAICKER. 1970. Adenohypophysial transmembrane potential: polarity reversal by elevated external potassium ion concentration. Science (Wash. D.C.). 167:182–184.

7. ORCI, L., M. AMHERDT, J. C. HENQUIN, A. E. LAMBERT, R. ÜBER, and A. E. REINOLD. 1973. Pronase effect on pancreatic β cell secretion and morphology. Science (Wash. D.C.). 180:647–649.

8. SCHOFIELD, J. G. 1967. Measurement of growth hormone released by ox pituitary slices in vitro. Biochem. J. 103:331–341.

9. SCHOFIELD, J. G., and E. N. COLE. 1971. Behaviour of systems releasing ox growth hormone in vitro. Mem. Soc. Endocrinol. 19:185–199.

10. SCHOFIELD, J. G., and M. MCPHERSON. 1974. Increase in pituitary adenosine 3':5'-cyclic monophosphate content and potentiation of growth hormone release from heifer anterior pituitary slices incubated in the presence of 3-isobutyl-1-methylxanthine. Biochem. J. 142:295–300.

11. SCHOFIELD, J. G., and M. STEAD. 1971. ATP, calcium uptake, and growth hormone release. FEBS (Fed. Eur. Biochem. Soc.) Lett. 13:149–151.

12. WALLACH, D. F. H. 1972. The disposition of proteins in the plasma membranes of animal cells: analytical approaches using controlled peptidolysis and protein labels. Biochim. Biophys. Acta. 265:61–83.

TANNIC ACID-STAINED MICROTUBULES WITH 12, 13, AND 15 PROTOFILAMENTS

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Subunit structure in the walls of sectioned microtubules was first noted by Ledbetter and Porter (6), who clearly showed that certain microtubules of plant meristematic cells have 13 wall protofilaments when seen in cross section. Earlier, protofilaments of microtubular elements had been described in negatively stained material, although exact counts of their number were difficult to