expected. However, it was confirmed by measuring the accumulation of enzymically
assayable ammonia. The very high $K_i$ for $\alpha$-cyano-4-hydroxycinnamate measured under
these conditions is also consistent with an acid matrix. The value of $6 \mu M$ reported pre-
viously (Halestrap, 1975) was determined under conditions where the mitochondrial
matrix was more alkaline than the medium. Addition of valinomycin to mitochondria
metabolizing pyruvate caused the matrix to become alkaline, and pyruvate metabolism
was increased considerably.

The results in the present paper are consistent with an action of glucagon on the
mitochondrial pH gradient. Consistent with this finding is the observation that
glucagon treatment of the perfused liver may increase the mitochondrial content of anionic
metabolites (Parrilla et al., 1975) and that glucagon causes a stimulation of the
phosphorylation of the mitochondrial membrane (Zahlten et al., 1972).

During the course of this work I was a Beit Memorial Research Fellow.

Mechanism of Action of Somatostatin: Growth-Hormone Release,
$[^{45}Ca]$Calcium Ion Efflux and Cyclic Nucleotide Metabolism of
Bovine Anterior-Pituitary Slices in the Presence of Prostaglandin E$_2$
and 1-Methyl-3-isobutylxanthine

R. J. BICKNELL,* P. W. YOUNG,* J. G. SCHOFIELD* and JANET ALBANO†

*Department of Biochemistry, and †Department of Medicine, Medical School,
University of Bristol, University Walk, Bristol BS8 1TD, U.K.

Several lines of evidence indicate that the increase in growth-hormone release observed
in vitro in the presence of prostaglandins and methylxanthines, dibutyryl cyclic AMP and
hypothalamic extracts presumed to contain the growth-hormone-releasing factor, is
due to an increase in the intracellular concentration of cyclic AMP. Thus the ability of
prostaglandins and hypothalamic extracts to stimulate growth-hormone release is en-
hanced in the presence of methylxanthine phosphodiesterase inhibitors, and the rise in
cyclic AMP concentration apparently precedes the release of growth hormone (Steiner
et al., 1970; Schofield & McPherson, 1974). Some additional evidence indicates that a
redistribution of intracellular Ca$^{2+}$ mediates this increased growth-hormone release.
Thus the increase in hormone release, but not cyclic AMP concentration, is inhibited by
deployment of tissue Ca$^{2+}$, although verapamil (an inhibitor of Ca$^{2+}$ entry into cells) does
not inhibit the hormone release (Eto et al., 1974).

It has been suggested that somatostatin, a peptide isolated from sheep hypothalami
and capable of inhibiting growth-hormone secretion, acts by modifying the cyclic nucleo-
tide response in the pituitary. In support of this suggestion, it has been shown in rat
pituitaries that somatostatin decreases the rise in cyclic AMP in the presence of prostag-
landin and theophylline (Borgeat et al., 1974), and that somatostatin increases the
concentration of cyclic GMP (Kaneko et al., 1974). We here report that somatostatin,
In (a) after 22 min perfusion, prostaglandin E$_2$ (1 $\mu$M) and 1-methyl-3-isobutylxanthine (0.5 mM) were introduced in the absence ($\square$) or presence ($\equiv$) of somatostatin (1 $\mu$g/ml). In (b) and (c) after 22 min of perfusion, prostaglandin E$_2$ (1 $\mu$M) and 1-methyl-3-isobutylxanthine (0.5 mM) were introduced in the absence (b) or presence (c) of somatostatin (1 $\mu$g/ml). Each point is the mean of three observations.

at a concentration which blocks stimulation of growth-hormone release by prostaglandin and 1-methyl-3-isobutylxanthine, does not modify the associated changes in ox pituitary cyclic AMP and cyclic GMP concentrations, $^{45}$Ca$^{2+}$ efflux, or protein kinase activity.

**Experimental**

Bovine anterior-pituitary slices were prepared and incubated in Krebs-Ringer bicarbonate-buffered salt solution as previously described (Schofield & McPherson, 1974). For determination of the effects of prostaglandins and somatostatin on growth-hormone release and $^{45}$Ca$^{2+}$ efflux, slices were incubated for 4 h in 1 ml of incubation medium containing 20 $\mu$Ci of $^{45}$Ca$^{2+}$ (0.8 mCi/mm), and then perfused at 37°C in incubation medium containing 25 mM-CaCl$_2$; 2 min (400 $\mu$l) fractions were collected. Growth hormone was measured by radioimmunoassay. For determination of cyclic AMP and cyclic GMP concentrations, tissue was frozen between aluminium blocks cooled in liquid N$_2$, denatured at 100°C, homogenized and deproteinized in acid/ethanol as described by Albano *et al.* (1976). Extracts were dried, the residues resuspended in Tris/HCl (50 mM, pH 7.4), and cyclic AMP was measured by a protein-binding assay (Brown *et al.*, 1971), and cyclic GMP by using rabbit antiserum raised to succinylated cyclic GMP conjugated to human serum albumin. For measurement of protein kinase activity, the tissue (70–100 mg) was homogenized at 0°C in potassium phosphate (20 mM, pH 6.5), containing EDTA (1 mM) and either sucrose (0.25 M) or NaCl (0.4 M), by using a Polytron homogenizer (10 s, position 3). The homogenate was centrifuged (Eppendorf 3200, 2 min), and protein kinase activity was measured in the supernatant with histone II AS (Sigma) as substrate and quenching the reaction as described by Wastila *et al.* (1971).

**Results and discussion**

Addition of prostaglandin E$_2$ (1 $\mu$M) and 1-methyl-3-isobutylxanthine (0.5 mM) to the perfusion medium caused a rapid increase in growth-hormone release (Fig. 1a). At the
same time, a transient increase in the efflux of $^{45}\text{Ca}^{2+}$ was observed (Fig. 1b). Since the external $\text{Ca}^{2+}$ concentration (2.5 mM) would be expected to be greater than the internal free $\text{Ca}^{2+}$ concentration, $^{45}\text{Ca}^{2+}$ efflux probably represents isotope exchange due possibly to changes in cell-membrane permeability or to redistribution of tissue $\text{Ca}^{2+}$. Somatostatin (1 $\mu$g/ml) completely blocked the release of growth hormone (Fig. 1a), but did not modify the transient efflux of $^{45}\text{Ca}^{2+}$. This efflux therefore does not represent $^{45}\text{Ca}^{2+}$ contained within growth-hormone secretory granules. Moreover, if efflux of $^{45}\text{Ca}^{2+}$ and growth-hormone release are both consequences of an action of prostaglandin and isobutylmethylxanthine on $\text{Ca}^{2+}$ translocation, the data suggest that somatostatin acts on secretion at a later stage.

Addition of isobutylmethylxanthine to pituitary slices increased tissue cyclic GMP 8-fold over 30 min (0.07–0.58 pmol/mg wet weight) and increased cyclic AMP 14-fold (0.16–2.31 pmol/mg wet weight). Somatostatin (1 $\mu$g/ml) did not modify cyclic nucleotide concentrations in the presence or absence of isobutylmethylxanthine (Fig. 2). The protein kinase activity ratio [for definition see Corbin et al. (1973)] was increased from 0.58±0.5 to 0.88±0.06 ($n=7$) after 10 min incubation in prostaglandin E$_2$ (1 $\mu$g/ml) and isobutylmethylxanthine (0.5 mM); somatostatin (1 $\mu$g/ml) did not modify either the basal or the stimulated activity.

The inability of somatostatin to modify changes in $\text{Ca}^{2+}$ distribution, cyclic nucleotide metabolism or protein kinase activity at a concentration which blocks the increase in growth-hormone release in response to prostaglandins and isobutylmethylxanthine strongly suggests that the peptide acts at a late stage in the secretory process.

This work is supported by a grant from the Medical Research Council, and P. W. Y. holds a Medical Research Council Scholarship. We also thank Dr. R. Guillem in for the gift of synthetic linear somatostatin.
Adenylate Cyclase in Plasma Membranes Purified from Rat Osteogenic Sarcoma

AILEEN CRAWFORD, RODERICK COLLIN, NICHOLAS H. HUNT and T. JOHN MARTIN

Department of Chemical Pathology, University of Sheffield Medical School, Sheffield S10 2RX, U.K.

An osteogenic sarcoma has been induced in Sprague–Dawley rats by intraperitoneal injection of [32P]orthophosphate (Bensted et al., 1961) and has been maintained by serial subcutaneous transplantation into syngeneic hosts as previously described (Martin et al., 1976). Partially purified plasma-membrane preparations from the transplanted tumour contained an adenylate cyclase, which was responsive in a dose-dependent manner to prostaglandins E\textsubscript{1} and E\textsubscript{2} and to bovine parathyroid hormone (Martin et al., 1976). Salmon calcitonin was not effective in that system.

Plasma membranes have been further purified by differential centrifugation. The tumour was excised and then homogenized in 1 mM-NaHCO\textsubscript{3} (pH 7.4, 4°C) buffer. The homogenate was centrifuged in the SS34 rotor of a Sorval RC2B centrifuge at 4°C. This rotor was used at this temperature for all centrifugation steps subsequently described. The centrifuge was switched on, allowed to reach 480g and then immediately slowed with the brake applied. The supernatant was re-centrifuged at 2200g for 10 min. The resultant pellet was resuspended in 2.5 ~sucrose/50mM-Tris/HCl, pH 7.4, to give a final sucrose concentration of 65% (w/v). This suspension was centrifuged at 9800g for 30 min. The supernatant was decanted and diluted to 48% (w/v) sucrose and re-centrifuged at 27000g for 30 min. The pellet was resuspended in 0.25m-sucrose/50mM-Tris/HCl/1 mM-EDTA buffer, pH 7.4. Membranes were stored in portions at −70°C after addition of dimethyl sulfoxide to a final concentration of 10% (v/v). To indicate the degree of purity of the plasma-membrane preparation, the following marker-enzyme assays were performed: 5’-ribonucleotidase (Mitchell & Hawthorne, 1965), cytochrome oxidase (Sottocasa et al., 1967), NADH oxidase (Avruch & Wallach, 1971), alkaline phosphatase (Lowry et al., 1954) and succinate dehydrogenase (Pennington, 1961). Protein concentrations were measured by the method of Kalckar (1947) or by the Hartree (1972) modification of the method of Lowry et al. (1951).

Plasma membranes prepared by this method contained adenylate cyclase, which was responsive to bovine parathyroid hormone in a dose-dependent manner, half-maximal activation occurring at 0.1 μM-parathyroid hormone. The GTP analogue guanylyl-5’-y lidimidodiphosphate [GMP-P(NH)P] also stimulated plasma-membrane adenylate cyclase with half-maximal activation occurring at μM. Prostaglandins E\textsubscript{1} and E\textsubscript{2} also activated adenylate cyclase, but salmon calcitonin had no effect.