Calcium-Dependent Inhibition of Protein Synthesis in Rat Parotid Gland

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1. Protein synthesis in the rat parotid gland *in vitro* was studied by measuring the incorporation of [^3H]phenylalanine into trichloroacetic acid-insoluble proteins. In the unstimulated gland, the rate of incorporation was dependent on the phenylalanine concentration in the medium and proceeded linearly for up to 3 h. 2. Adrenaline, carbamoylcholine, phenylephrine and ionophore A23187 inhibited the incorporation of [^3H]phenylalanine into acid-insoluble protein; isoprenaline, dibutyl cyclic AMP and 8-bromo-cyclic GMP were inactive. 3. Inhibition by adrenaline and carbamoylcholine but not by ionophore A23187 required extracellular Ca^{2+} . 4. Both adrenaline and carbamoylcholine increased the magnitude of the acid-soluble [^3H]phenylalanine pool at $10\text{ }\mu\text{M}$ extracellular phenylalanine, but had no effect if the phenylalanine concentration was increased to $200\text{ }\mu\text{M}$. 5. There was no correlation between cellular ATP content and the observed inhibition of protein synthesis. 6. Our results suggest that both α -adrenergic and cholinergic receptors may play a role in the regulation of protein synthesis in the rat parotid gland, and that their effects are mediated by a rise in intracellular free Ca^{2+} .

Parotid-gland metabolism is subject to differential control by both adrenergic and cholinergic receptors (for review see Schramm & Selinger, 1975). Activation of the β -adrenergic receptor stimulates adenylate cyclase (Butcher *et al.*, 1975; Wojcik *et al.*, 1975) and induces a rapid and copious release of amylase (Bdolah *et al.*, 1964; Butcher *et al.*, 1975; Mangos *et al.*, 1975). Moreover, the incorporation of radioactively labelled amino acids into trichloroacetic acid-insoluble proteins and amylase is also increased 4–6 h after β -adrenergic stimulation (Lillie & Han, 1973; Johnson & Sreebny, 1973). These findings have led to the suggestion that cyclic AMP serves as the second messenger for both biosynthesis and release of secretory protein. The reason for the temporal dissociation between the onset of secretion and protein biosynthesis, however, remains obscure.

α -Adrenergic and cholinergic agonists activate guanylate cyclase (Wojcik *et al.*, 1975) and induce K^+ release (Batzri *et al.*, 1973; Selinger *et al.*, 1973). Secretion of amylase is also elevated by these agonists, albeit weakly in comparison with β -adrenergic agonists (Babad *et al.*, 1967; Mangos *et al.*, 1975; Kanagasuntheram & Randle, 1976). These effects are dependent on extracellular Ca^{2+} and are believed to be initiated by a rise in intracellular free Ca^{2+} (Selinger *et al.*, 1973; Kanagasuntheram & Randle, 1976). Consistent with this hypothesis, the ionophore A23187, which promotes Ca^{2+} influx into cells, elevates cyclic GMP concentration and stimulates release of K^+ and amylase from parotid glands *in vitro* (Selinger *et al.*, 1974; Butcher, 1975). The effects of α -adrenergic or cholinergic stimulation on

parotid-gland secretory-protein biosynthesis have not been investigated.

In the present study we have tried to evaluate the role of adrenergic and cholinergic receptors in regulating protein synthesis in the rat parotid gland. The action of various pharmacologically active compounds and secretagogues on the incorporation of radioactively labelled amino acid into protein has been determined *in vitro*. We have also investigated the involvement of Ca^{2+} in these receptor-mediated responses.

Experimental

Materials

Adrenaline, isoprenaline, phenylephrine, carbamoylcholine, propranolol, ATP, DNA (type I), β -hydroxybutyric acid, L-amino acids and bovine serum albumin (fraction V) were from Sigma Chemical Co., St. Louis, MO, U.S.A. Phentolamine mersylate was from Ciba Laboratories, Horsham, West Sussex, U.K. NCS tissue solubiliser was obtained from Amersham/Searle Corp., Arlington Heights, IL, U.S.A. Ionophore A23187 was a gift from Dr. R. L. Hamill, Eli Lilly and Co., Indianapolis, IN, U.S.A. All other chemicals were of the purest grade available. [$2,3\text{-}^3\text{H}$]Phenylalanine and [$\text{U-}^{14}\text{C}$]sucrose were from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation and incubation of parotid slices

We commenced all experiments at about the same time each day to avoid changes in the rate of protein

synthesis caused by diurnal variation (Sreebny *et al.*, 1971). Parotid glands were removed from albino Wistar rats (approx. 200–250g body wt.) which had free access to food and water. The glands were cleaned and sliced as previously described (Kanagasuntheram & Randle, 1976) in bicarbonate-buffered medium (Krebs & Henseleit, 1932) supplemented with 5 mM-glucose, 5 mM- β -hydroxybutyrate, 1 mg of bovine serum albumin/ml and an MEM (minimal essential medium) amino acid supplement (Eagle, 1959) containing 10 μ M-L-phenylalanine. The medium was gassed with O₂/CO₂ (19:1) and the slices were preincubated for 10–20 min at 37°C, with shaking at 60 cycles/min. The medium was decanted and immediately replaced with fresh medium of similar composition containing 0.5 μ Ci of [2,3-³H]-phenylalanine/ml and other additions as described in the text, or in legends to Figures and Tables. After incubation for 30 min, the tissue was homogenized in 1.5 ml of water with a Polytron PT-10 homogenizer, and the homogenate used for the measurement of DNA and of [³H]phenylalanine incorporation. When incorporation into the trichloroacetic acid-soluble fraction was also determined, the tissue slices were first washed three times with ice-cold medium lacking [³H]phenylalanine, before homogenization. 80–85% of the extracellular phenylalanine was removed by the washing procedure. The extracellular space in parotid pieces was estimated in separate experiments with [U-¹⁴C]sucrose used as an extracellular marker. To determine the time course of agonist activity, parotid pieces were incubated as described above for up to 3 h, except that [2,3-³H]-phenylalanine was omitted from the incubation medium. At 30 min before the end of the experiment, the medium was replaced with similar medium containing radioactive phenylalanine (0.5 μ Ci/ml).

Analytical methods

Incorporation of [³H]phenylalanine into trichloroacetic acid-insoluble or -soluble fractions was determined as described by Jamieson & Palade (1971). Tissue homogenates were precipitated with ice-cold 10% (w/v) trichloroacetic acid and the acid-soluble and -insoluble fractions separated by centrifugation at 3000g for 5 min. The precipitated acid-insoluble protein was washed twice with 10% (w/v) trichloroacetic acid and solubilized in NCS tissue solubilizer. Samples were counted for radioactivity in toluene-based scintillation mixture containing 2,5-diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene. Correction for quenching was by the channels-ratio method.

ATP, DNA and protein were measured as described previously (Kanagasuntheram & Randle, 1976).

Results

In preliminary experiments, trichloroacetic acid-insoluble radioactivity of both tissue and incubation medium was determined to ascertain whether newly synthesized protein was discharged into the medium. After 30 min of incubation, acid-insoluble radioactivity in the medium constituted less than 4% of the total incorporation into control or stimulated slices. In view of the very low concentration of labelled secretory material in the medium, radioactivity associated with this fraction was not determined in any of the subsequent experiments.

The relationship between phenylalanine concentration in the medium and its incorporation into acid-insoluble and acid-soluble material by rat parotid slices is shown in Fig. 1. Radioactivity incorporated into the acid-insoluble fraction was always significantly greater than that of the acid-soluble fraction, which showed a much slower increase in pool size with increasing phenylalanine concentration. Although 50 μ M-phenylalanine was sufficient to sustain almost maximal rates of incorporation into proteins, incorporation into the acid-soluble pool was only 24% of that obtained at 200 μ M-phenylalanine. At 10 μ M-phenylalanine, incorporation into acid-in-

Fig. 1. Incorporation of [³H]phenylalanine into trichloroacetic acid-insoluble and -soluble fractions at different phenylalanine concentrations

Parotid slices were incubated for 30 min in supplemented bicarbonate-buffered medium containing 1–200 μ M-phenylalanine and 0.5 μ Ci of [³H]phenylalanine/ml. Incorporation into the acid-soluble (○) and -insoluble (●) fractions was determined as described in the Experimental section. Each point is the mean of four or more determinations.

Fig. 2. Time course of inhibition of $[^3\text{H}]$ phenylalanine incorporation into the trichloroacetic acid-insoluble fraction by adrenaline and carbamoylcholine

Parotid pieces were incubated in supplemented bicarbonate medium (see the Experimental section) containing $10\text{ }\mu\text{M}$ -adrenaline (●) or $10\text{ }\mu\text{M}$ -carbamoylcholine (○). $[^3\text{H}]$ Phenylalanine ($0.5\text{ }\mu\text{Ci/ml}$) was added to the incubation medium for the last 30 min of incubation. $[^3\text{H}]$ Phenylalanine incorporation into acid-insoluble proteins was calculated as nmol/30 min per mg of DNA. Inhibition (%) = $100[(\text{control rate} - \text{stimulated rate})/\text{control rate}]$. Each point is the mean of four or more determinations.

Fig. 3. Effect of adrenaline concentration and carbamoylcholine concentration on the inhibition of $[^3\text{H}]$ phenylalanine incorporation into the trichloroacetic acid-insoluble fraction

Parotid pieces were incubated in medium containing adrenaline (●) or carbamoylcholine (○), and incorporation of $[^3\text{H}]$ phenylalanine was determined as described in the Experimental section. Results, calculated as described in Fig. 2, are the means of three or more experiments.

soluble proteins proceeded at a linear rate of $0.87 \pm 0.09\text{ nmol/min}$ per mg of DNA for up to 3 h. Fig. 2 shows the effect of adrenaline and carbamoylcholine on the rate of incorporation into the trichloroacetic acid-insoluble fraction. Both agonists inhibited incorporation, the inhibition being maximal during the first 30 min of incubation with the agonist. In carbamoylcholine-treated pieces, the rate returned to control values by 3 h; with adrenaline, despite a partial reversal of inhibition, the rate remained significantly lower even after 3 h. It appeared that with prolonged incubation in the presence of either adrenaline or carbamoylcholine, the tissue became increasingly insensitive to the agonist, for addition of fresh adrenaline or carbamoylcholine at various times during the 3 h incubation had no effect on the observed reversal of inhibition.

Inhibition by adrenaline and carbamoylcholine was concentration dependent (Fig. 3), but adrenaline was a more potent inhibitor than carbamoylcholine at the concentrations tested; $10\text{ }\mu\text{M}$ -adrenaline lowered the rate of incorporation by 53%, compared with

31% by the same concentration of carbamoylcholine.

A decrease in the rate of incorporation of phenylalanine into protein may arise from an inhibition of either amino acid transport or protein synthesis itself. To establish which of these two processes was subject to regulation, we determined the effect of adrenaline and carbamoylcholine on the incorporation of $[^3\text{H}]$ phenylalanine into the acid-soluble fraction. Radioactivity of this fraction was greater in adrenaline- and carbamoylcholine-stimulated slices at low phenylalanine concentration ($10\text{ }\mu\text{M}$), but showed no significant difference from control slices if the phenylalanine concentration was increased to $200\text{ }\mu\text{M}$ (Table 1). These observations would be consistent with a scheme in which both agonists exert an inhibitory effect on the protein-synthetic mechanism, but not on amino acid transport. The assumption is made that stimulation does not alter the specific radioactivity of the intracellular trichloroacetic acid-soluble phenylalanine pool. Thus it is envisaged that, at low phenylalanine concentration, amino acid transport is rate-limiting with respect to

Table 1. *Effect of adrenaline and carbamoylcholine on [³H]phenylalanine incorporation into the trichloroacetic acid-soluble fraction at different phenylalanine concentrations*

Parotid pieces were incubated in bicarbonate-buffered medium containing either 10 μ M- or 200 μ M-phenylalanine, 0.5 μ Ci of [³H]phenylalanine/ml and other additions as indicated. [³H]Phenylalanine incorporation into the acid-soluble fraction was determined as described in the Experimental section. Results are the means $\pm$ S.D. of the numbers of observations shown in parentheses. **P* < 0.01 for difference from control.

Experiment	Phenylalanine concn. (μ M)	[³ H]Phenylalanine incorporated (nmol/mg of DNA)
Control	10	0.53 $\pm$ 0.09 (4)
Adrenaline (10 μ M)	10	1.03 $\pm$ 0.14 (4)*
Carbamoylcholine (10 μ M)	10	0.98 $\pm$ 0.23 (4)*
Control	200	32.8 $\pm$ 4.9 (6)
Adrenaline (10 μ M)	200	35.9 $\pm$ 4.0 (6)
Carbamoylcholine (10 μ M)	200	34.5 $\pm$ 7.0 (6)

Table 2. *Effects of adrenaline, isoprenaline, carbamoylcholine, phenylephrine, dibutyl cyclic AMP and 8-bromo-cyclic GMP on the incorporation of [³H]phenylalanine into trichloroacetic acid-insoluble protein and on ATP content*

Incorporation of [³H]phenylalanine into acid-insoluble proteins and ATP concentrations were determined in separate experiments. Incubations were carried out as described in the Experimental section in supplemented bicarbonate medium containing additions as shown below. In experiments with phentolamine or propranolol, the blocking agent was also present in the preincubation medium. Results are the means $\pm$ S.D. of the numbers of observations shown in parentheses. **P* < 0.01 for difference from appropriate control.

Experiment	[³ H]Phenylalanine incorporated (nmol/mg of DNA)	ATP (nmol/mg of protein)
Control	24.4 $\pm$ 1.8 (10)	4.07 $\pm$ 0.42 (6)
Isoprenaline (10 μ M)	25.4 $\pm$ 2.1 (6)	1.79 $\pm$ 0.21 (6)*
Adrenaline (10 μ M)	11.7 $\pm$ 2.5 (9)*	1.41 $\pm$ 0.07 (6)*
Adrenaline (10 μ M) + phentolamine (10 μ M)	23.2 $\pm$ 2.3 (6)	—
Adrenaline (10 μ M) + propranolol (10 μ M)	17.1 $\pm$ 2.4 (6)*	—
Phenylephrine (10 μ M)	25.0 $\pm$ 2.5 (6)	2.19 $\pm$ 0.34 (6)*
Phenylephrine (100 μ M)	17.1 $\pm$ 1.9 (6)*	—
Phenylephrine (10 μ M) + isoprenaline (10 μ M)	14.1 $\pm$ 1.9 (4)*	—
Phenylephrine (100 μ M) + isoprenaline (10 μ M)	11.3 $\pm$ 0.5 (4)*	—
Control	27.6 $\pm$ 2.5 (18)	4.07 $\pm$ 0.42 (6)
Carbamoylcholine (10 μ M)	19.4 $\pm$ 2.1 (13)*	1.37 $\pm$ 0.24 (6)*
Dibutyl cyclic AMP (1 mM)	27.8 $\pm$ 1.9 (6)	2.1 $\pm$ 0.14 (6)*
8-Bromo-cyclic GMP (0.1 mM)	27.1 $\pm$ 4.5 (8)	3.65 $\pm$ 0.25 (6)

protein synthesis. A lowering of the protein-synthetic rate would therefore increase the intracellular phenylalanine pool. In contrast, at high phenylalanine concentration, when the intracellular phenylalanine pool has already reached its near-maximal steady-state value, inhibition of protein synthesis would no longer be able to increase further the intracellular phenylalanine pool size.

The adrenaline-mediated inhibition of protein synthesis appeared to be an α -adrenergic effect in that it was reversed by phentolamine (an α -receptor blocker) but not propranolol (a β -receptor blocker) (Table 2). Propranolol did, however, partially reverse the adrenaline-induced inhibition. Mediation of inhibition by the α -receptor was further supported

by the observation that isoprenaline, a predominantly β -adrenergic effector, and dibutyl cyclic AMP, at concentrations which induce a large and rapid stimulation of amylase secretion *in vitro* (Babad *et al.*, 1967), had no effect on the incorporation of phenylalanine into proteins. The α -adrenergic agonist phenylephrine also inhibited protein synthesis, but only at relatively high concentrations (100 μ M). Addition of 10 μ M-isoprenaline together with phenylephrine, however, not only enhanced the inhibition of protein synthesis by 100 μ M-phenylephrine, but also lowered the threshold concentration for phenylephrine-mediated inhibition. Thus a combination of 10 μ M-phenylephrine and 10 μ M-isoprenaline decreased [³H]phenylalanine incorpor-

Table 3. *Effects of adrenaline, carbamoylcholine and ionophore A23187 on the incorporation of [³H]phenylalanine into trichloroacetic acid-insoluble proteins in the presence and absence of Ca²⁺*

Tissue slices were preincubated for 10 min in bicarbonate medium lacking calcium ($-Ca^{2+}$) or containing 2 mM- Ca^{2+} and subsequently incubated for 30 min in similar medium containing [³H]phenylalanine and other additions as shown. For experiments with ionophore A23187 in Ca^{2+} -free medium, 1 mM-EGTA was also present in the preincubation and incubation media. Each value is the mean $\pm$ S.D. of the number of observations shown in parentheses. * $P < 0.01$ for difference from appropriate control.

Experiment	[³ H]Phenylalanine incorporated (nmol/mg of DNA)	
	+ 2 mM- Ca^{2+}	- Ca^{2+}
Control	24.5 $\pm$ 1.9 (8)	22.5 $\pm$ 1.7 (4)
Adrenaline (10 μ M)	11.7 $\pm$ 2.5 (9)	21.5 $\pm$ 2.4 (4)
Control	27.6 $\pm$ 2.5 (18)	22.5 $\pm$ 1.7 (4)
Carbamoylcholine (10 μ M)	19.4 $\pm$ 2.1 (6)*	22.0 $\pm$ 1.6 (4)
Control	29.1 $\pm$ 2.7 (7)	28.8 $\pm$ 2.1 (4)
Ionophore A23187 (20 μ g/ml)	18.5 $\pm$ 2.0 (3)*	—
Ionophore A23187 (40 μ g/ml)	14.7 $\pm$ 1.8 (4)*	18.4 $\pm$ 1.7 (4)*

ation into acid-insoluble proteins by 42%, although neither agonist, at the same concentration, had any significant effect on the rate of incorporation when present on its own. These findings suggest that whereas the α -adrenergic receptor plays the primary role in turning off protein synthesis in the rat parotid gland, activation of the β -receptor can potentiate this α -receptor-mediated effect.

As shown in Table 2, 8-bromo-cyclic GMP had no effect on the rate of protein synthesis. Absence of inhibition with 8-bromo-cyclic GMP may not necessarily prove that cyclic GMP is not involved in the inhibitory process, for the ability of the 8-bromo derivative to substitute effectively for cyclic GMP is questionable. Indeed attempts to mimic other α -adrenergic or cholinergic effects in the parotid gland with cyclic GMP or its derivatives have met with only limited success (Selinger *et al.*, 1974; Lambert *et al.*, 1975).

Omission of Ca^{2+} from the incubation medium abolished the adrenaline- and carbamoylcholine-mediated inhibition (Table 3). This dependence on extracellular Ca^{2+} is shared by other α -adrenergic and cholinergic effects such as secretion of K^+ or amylase and it is believed that an increase in Ca^{2+} influx which accompanies such stimulation (Kanagasuntheram & Randle, 1976; Putney, 1976; Miller & Nelson, 1977) triggers these processes. That an increase in intracellular Ca^{2+} may also mediate inhibition of protein synthesis was supported by the observation that the bivalent-cation ionophore A23187 also decreased the incorporation of phenylalanine into the trichloroacetic acid-insoluble fraction. Dimethyl sulphoxide, the solvent in which the ionophore was dissolved, had no effect. Inhibition by ionophore A23187 was observed even when extracellular free Ca^{2+} was removed with 0.1 mM-EGTA. Besides permitting free movement of Ca^{2+} across the

plasma membrane, ionophore A23187 can also mobilize intracellular Ca^{2+} , and it is likely that the release of Ca^{2+} from these intracellular stores alone is sufficient to induce inhibition of protein synthesis.

In view of the ATP requirement for protein biosynthesis, it was necessary to study the relationship between cellular ATP concentration and the rate of protein synthesis. The ATP content of parotid pieces incubated for 30 min with various stimuli is shown in Table 2. At the concentrations used, adrenaline and carbamoylcholine lowered cellular ATP content by more than 50%; isoprenaline and dibutyryl cyclic AMP were slightly less effective. 8-Bromo-cyclic GMP had no effect. The difference in the ATP content of adrenaline- or carbamoylcholine-stimulated slices and isoprenaline- or dibutyryl cyclic AMP-stimulated slices was not significant, and may be taken as an indication that there is no correlation between cellular ATP content and inhibition of protein synthesis.

Discussion

The greater part of the protein-synthetic mechanism (75–80%) in rat parotid glands is directed towards secretory-protein biosynthesis. Studies *in vivo* by Sreebny *et al.* (1971) established that secretory protein synthesis is stimulated 6 h after induction of salivation by mastication. Moreover, this stimulatory effect appeared to be mediated by the β -adrenergic receptor, for isoprenaline, a β -adrenergic stimulant, also produced a similar pattern of accelerated protein synthesis (Johnson & Sreebny, 1973; Lillie & Han, 1973). In the present study we were unable to observe any increase in protein-synthetic rates after stimulation with isoprenaline or dibutyryl cyclic AMP. The relatively short incubation period used in the present experiments may have precluded detection of

stimulated synthesis, which becomes apparent only after 4 h (Lillie & Han, 1973).

Grand & Gross (1969), however, reported an increase in the specific radioactivity of trichloroacetic acid-insoluble proteins 30 min after stimulation of rat parotid slices with adrenaline or dibutyl cyclic AMP. This apparent stimulation may have been due to a substantial decrease in total protein of the gland brought about by discharge of secretory products. To circumvent this possible source of error, we expressed incorporation in terms of DNA content. Our observations indicate that immediately after stimulation with either adrenaline or carbamoylcholine, protein synthesis in the rat parotid gland is inhibited. These findings *in vitro* are in agreement with those of Gromet-Elhanan & Winnick (1963) that protein synthesis in the rat parotid gland *in vivo* falls to a minimum about 1 h after initiation of secretion with food and pilocarpine.

In the guinea-pig exocrine pancreas incubated *in vitro*, Jamieson & Palade (1971) found no significant change in protein synthesis after carbamoylcholine stimulation. Bauduin *et al.* (1973), however, reported a marked inhibition of protein synthesis in rat pancreatic slices incubated with carbamoylcholine, pancreozymin and caruelin. The inhibition occurred very early in the course of action of the secretagogue and persisted for up to 100 min.

Conditions which deplete the energy resources of the cell inhibit protein synthesis (Jamieson & Palade, 1968; Bauduin *et al.*, 1969). Bauduin *et al.* (1973) have speculated on the existence of an indirect link between secretion and protein synthesis such that the channelling of cellular energy towards accelerated secretion deprives the protein-synthetic machinery of ATP and thereby inhibits protein biosynthesis. If such a link does indeed exist in the parotid gland, it must be assumed that cellular ATP is compartmentalized such that isoprenaline or dibutyl cyclic AMP and α -adrenergic agonists or carbamoylcholine, mobilize separate cellular ATP pools. Otherwise it is difficult to account for the absence of inhibition in isoprenaline- or dibutyl cyclic AMP-stimulated slices.

In common with other α -adrenergic and cholinergic effects, inhibition of protein synthesis required extracellular Ca^{2+} and was probably mediated by a rise in intracellular free Ca^{2+} . β -Adrenergic activation may also increase intracellular free Ca^{2+} by releasing Ca^{2+} from intracellular Ca^{2+} pools (Dormer & Ashcroft, 1974; Kanagasuntheram & Randle, 1976). Although this increase in free Ca^{2+} induced by β -adrenergic stimulation may in itself be insufficient to affect the rate of protein synthesis, it could account for the augmentation of the α -adrenergic effect, when both α - and β -receptors are activated simultaneously. Whether Ca^{2+} exerts a direct regulatory influence on protein synthesis, such as is postulated for stimulus-

secretion coupling (see review by Rubin, 1974), or acts indirectly by changing the concentration of intracellular messengers such as cyclic AMP and cyclic GMP, is not known, and further studies are necessary to establish its exact mode of action.

On the basis of earlier findings (Sreebny *et al.*, 1971; Johnson & Sreebny, 1973; Lillie & Han, 1973), as well as the present study, it may be concluded that protein synthesis in the rat parotid gland is subject to opposing regulatory influences by β -adrenergic receptors and α -adrenergic or cholinergic receptors. Although the former exert a temporally dissociated but prolonged stimulatory effect on protein biosynthesis, the latter induce a more immediate inhibitory effect on the process. It is conceivable that α -adrenergic and cholinergic stimulation, by inhibiting protein synthesis, exert a sparing effect on the cell's energy resources, thereby facilitating the utilization of ATP for other processes such as K^+ or enzyme secretion.

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