Mucin release and calcium fluxes in isolated rat submandibular acini

Margaret A. McPHERSON and Robert L. DORMER

Department of Medical Biochemistry, Welsh National School of Medicine, Heath Park,
Cardiff CF4 4XN, U.K.

(Received 22 June 1984/Accepted 13 August 1984)

1. A method is described for preparing isolated rat submandibular acini by collagenase digestion followed by mechanical dispersion. As assessed by Trypan Blue exclusion, phase contrast microscopy, ATP content and release of mucins and lactate dehydrogenase, the acini are morphologically and functionally intact. 2. Secretory function of isolated acini was similar to that of intact tissue in terms of time-course, dose dependence and degree of stimulation of mucin release by adrenergic secretagogues. 3. Mucin release was increased to the same extent (approx. 3–4-fold) by either isoproterenol or noradrenaline at a maximally effective concentration (10 μ M). 4. Stimulation of mucin release by isoproterenol (10 μ M), noradrenaline (10 μ M) or adrenaline (10 μ M) was inhibited by propranolol (30 μ M) but not by phentolamine (30 μ M). 5. Isoproterenol (10 μ M) increased both $^{45}\text{Ca}^{2+}$ uptake and efflux from the acini, which was shown to represent a net release of calcium. However, there was a delay (approx. 10 min) in onset of stimulation of $^{45}\text{Ca}^{2+}$ mobilization which was not apparent in isoproterenol stimulation of mucin release. 6. Our results indicate that increases in intracellular calcium mobilization in response to a β -adrenergic secretagogue do not trigger mucin secretion from rat submandibular acini.

The salivary glands are innervated by sympathetic and parasympathetic nerves which regulate secretion of water, electrolytes and proteins. The mechanism of secretion of serous proteins has been extensively studied *in vitro* using the rat parotid gland (Butcher & Putney, 1980), but less attention has been focused on studying secretion of mucins, an essential constituent of saliva.

The rat submandibular gland is seromucous, consisting predominantly of mucous acinar cells (Scott & Pease, 1959), which synthesize and secrete high- M_r mucins. This tissue therefore provides a good system in which to study the mechanisms controlling mucin secretion. The gland also contains granular duct cells which secrete proteinases (Matthews, 1974; Bogart & Picarelli, 1978); thus a preparation enriched in acinar cells would be advantageous for studying mucin release *in vitro*. Previous studies using tissue slices (Bogart & Picarelli, 1978) or dispersed acinar–intercalated duct complexes (Quissell & Redman, 1979; Quissell & Barzen, 1980) have confirmed evidence *in vivo* (Bogart, 1975; Martinez & Cassity, 1982) that

adrenergic agents are potent stimulators of mucin secretion. However, in tissue slices (Bogart & Picarelli, 1978) increases in mucin release were observed only in response to β -adrenergic stimulation, whereas in acinar–duct complexes (Quissell & Barzen, 1980) both α - and β -adrenergic stimulation of mucin release was reported.

Cyclic AMP and Ca^{2+} have been investigated as regulators of mucin release from rat submandibular glands (Quissell & Barzen, 1980; Quissell *et al.*, 1981), but their relative roles in the stimulation of mucin secretion is not clear. Experiments *in vivo* suggest that Ca^{2+} is released from rat submandibular glands in response to β -adrenergic stimulation (Dreisbach, 1967; Yu & Schneyer, 1983), but whether this is due to a direct action of β -agonists on the acinar cells is unknown.

In the present study, preparations of isolated rat submandibular acini have been developed, which have similar adrenergic secretory responses to those of intact tissue. The actions of a β -adrenergic secretagogue on $^{45}\text{Ca}^{2+}$ fluxes have been examined to assess the role of intracellular calcium move-

ments in the stimulation of mucin secretion. A preliminary account of some of these results has been reported (McPherson & Dormer, 1984a).

Experimental

Materials

D-(+)-[1-¹⁴C]Glucosamine (54 mCi/mmol) and ⁴⁵CaCl₂ (10–40 mCi/mg of calcium) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The following were purchased from Sigma: soya bean trypsin inhibitor (type 1-S), hyaluronidase (type 1), L-isoproterenol hydrochloride, L-adrenaline, L-noradrenaline, DL-propranolol. Adrenaline and noradrenaline were initially dissolved in 0.1 M-HCl. Phentolamine mesylate [2-N-(3-hydroxyphenyl)-p-toluidinomethyl-2-imidazoline-mesylate; Rogitine] was from Ciba, Horsham, Sussex, U.K. Collagenase was purchased from Millipore (Worthington type II). Bovine serum albumin (fraction V) was from Miles Laboratories, Stoke Poges, Bucks., U.K. and minimal Eagle's medium (MEM) essential amino acid supplement was from Gibco, Paisley, Scotland, U.K. Scintillation fluid was a xylene-based scintillation cocktail (Packard emulsifier scintillator 299). Nuclepore polycarbonate filters were from Nuclepore Corp., Pleasanton, CA, U.S.A. All other reagents, from BDH, were of the highest purity available.

Methods

Submandibular tissue. Submandibular glands were dissected from male Wistar rats (250–280 g) that had been starved overnight and killed by stunning and decapitation. Approx. 1 g wet wt. of tissue was obtained from two rats.

Incubation medium. The basic incubation medium (KHB) was a Krebs–Henseleit (1932) bicarbonate-buffered salt solution, containing 111 mM-NaCl, 4.7 mM-KCl, 1.0 mM-Na₂HPO₄, 1.13 mM-MgCl₂, 1.28 mM-CaCl₂, 32.5 mM-NaHCO₃, MEM amino acids, 1.2 mM-glucose, soya bean trypsin inhibitor (0.01%) and gassed with O₂/CO₂ (19:1), pH 7.4.

Preparation of tissue fragments. Tissue (1–2 g wet wt.) was placed in KHB and cut with scissors into small fragments weighing 25–40 mg wet wt. Fragments from four glands were preincubated in KHB (8–10 ml) containing 1% (w/v) bovine serum albumin for 15 min (shaking at 60 cycles/min) at 37°C.

Preparation of acini. Submandibular acini were prepared by a modification of our method (Williams *et al.*, 1978) for preparing pancreatic acini.

Tissue (1–2 g wet wt.) was injected with 5–10 ml of Ca²⁺- and Mg²⁺-free Hanks' balanced salt solution (Gibco), containing collagenase (64

units/ml) and hyaluronidase (500 units/ml). The tissue was dissected to remove the major ducts, cut into small fragments and incubated in the collagenase solution for 60 min at 37°C (shaking at 120 cycles/min), with the solution being replaced by similar, fresh solution after 10 min. The tissue was then mechanically dispersed by pipetting eight to ten times through a 2.4 mm diameter polypropylene tip attached to an adjustable Finnpiptette. Acini were filtered through two layers of cheese-cloth and purified by centrifugation (4 min, 50 g) through KHB containing 4% (w/v) bovine serum albumin. Acini were washed once in 10 ml of KHB containing 2% (w/v) bovine serum albumin and preincubated (shaking at 60 cycles/min) in 8 ml of similar medium approx. 10⁶ acini/ml) for 15 min at 37°C.

Release of mucins. Incubation of submandibular acini was carried out in medium containing 2% (w/v) bovine serum albumin, since our initial experiments (results not shown) showed that this concentration was required to prevent cell clumping and maintain viability. In initial experiments with tissue fragments, similar secretory responses were observed in either 1% or 2% (w/v) bovine serum albumin (results not shown); thus the lower concentration of albumin was used.

Mucin release was measured as previously described (McPherson *et al.*, 1983). After the preincubation period, D-(+)-[1-¹⁴C]glucosamine (1 μCi/ml) was added to incubation flasks containing acini or tissue fragments in KHB, for 90 min at 37°C. Acini or fragments were then washed three times with 10 ml of KHB containing glucosamine (1 mM), glucose (12 mM) and bovine serum albumin (2%, w/v, for acini; 1%, w/v, for fragments) and incubated in similar medium for 60 min at 37°C. Medium was then removed and acini (approx. 2 × 10⁵/ml) or fragments (70–100 mg wet wt./2.5 ml) were incubated under experimental conditions. At time zero and after the times stated in the text and Figure legends, aliquots of incubation medium were centrifuged (10 s in an Eppendorf microcentrifuge) and the mucins in 0.5 ml of medium were precipitated by addition of an equal volume of a trichloroacetic acid (20%, w/v)/phosphotungstic acid (1%, w/v) solution at 4°C. The precipitates were washed three times with a solution of trichloroacetic acid (10%, w/v)/phosphotungstic acid (0.5%, w/v) at 4°C and dissolved in 0.5 ml of 0.5 M-NaOH. The radioactivity in the samples was measured on the ¹⁴C channel of a Packard Tri-Carb liquid-scintillation counter with an efficiency of 80–85%. In all cases, quench corrections were made by an internal channels ratio method.

Release of lactate dehydrogenase. Acini were incubated under the same conditions as for

measurement of mucin release. After centrifugation (10s in an Eppendorf microcentrifuge) lactate dehydrogenase in the medium was measured spectrophotometrically.

Measurement of ATP content. The ATP concentration in neutralized HClO_4 extracts of tissue fragments or isolated acini was measured as described previously (McPherson & Schofield, 1974; Dormer, 1984), using the firefly luciferin-luciferase assay.

Measurement of $^{45}\text{Ca}^{2+}$ fluxes. The methods used for measurement of $^{45}\text{Ca}^{2+}$ uptake and efflux in isolated acini have been described previously (Dormer *et al.*, 1981).

$^{45}\text{Ca}^{2+}$ uptake was measured as follows. Acini were preincubated for 60min and then resuspended (approx. 3×10^5 acini/ml) in fresh KHB containing CaCl_2 (1.28mM), glucose (12mM) and bovine serum albumin (2%, w/v). After 15min equilibration at 37°C (shaking at 60 cycles/min), $^{45}\text{CaCl}_2$ (3 $\mu\text{Ci/ml}$), in the presence or absence of secretagogue, was added. Duplicate samples (0.4 or 0.5ml containing 0.3–0.6mg of protein) were taken from each flask at various times after $^{45}\text{Ca}^{2+}$ addition.

$^{45}\text{Ca}^{2+}$ efflux was measured as follows. Acini were preincubated for 60min and then resuspended (approx. 3×10^5 acini/ml) in fresh KHB containing CaCl_2 (1.28mM), glucose (12mM) and bovine serum albumin (2%, w/v). They were then labelled with $^{45}\text{CaCl}_2$ (3 $\mu\text{Ci/ml}$) for 60min at 37°C, centrifuged (2min, 50g), washed in ice-cold KHB and resuspended in fresh, non-radioactive medium at 37°C. Acini were divided between flasks, duplicate samples (0.4 or 0.5ml containing 0.3–0.6mg of protein) were taken immediately (time zero) and addition of secretagogues was made. Samples were then taken from each flask, in duplicate, at various times thereafter.

In both uptake and efflux experiments, acini were separated from the medium by dilution into 20 vol. of ice-cold saline (0.9%, w/v) and immediate filtration on Nuclepore filters (3 μm pore size). The filters were washed with a further 10 vol. of ice-cold saline (0.9%, w/v) and placed in plastic tubes. Water (2ml) was added and the mixture was sonicated for 5–10s (18 μm peak-to-peak using an MSE ultrasonic disintegrator). The radioactivity in 1ml aliquots of sonicated acini was measured on the ^{14}C channel of a Packard Tri-Carb liquid-scintillation counter with an efficiency of approx. 80%. Protein was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as standard. $^{45}\text{Ca}^{2+}$ content of the acini was expressed as nmol/mg of protein, based on the specific radioactivity of Ca^{2+} in the incubation medium.

Graphical analysis of $^{45}\text{Ca}^{2+}$ uptake curves. The

rate of $^{45}\text{Ca}^{2+}$ influx was determined by drawing tangents to the uptake curves at 2min intervals up to 10min and at 5min intervals thereafter, up to 120min. The log (rate) was plotted against time and the rate constants (min^{-1}) were derived from straight lines which gave the best fit by linear regression analysis (correlation coefficients of 0.92–0.99 were obtained for all values presented). The pool size, in nmol/mg of protein, was calculated from the flux at time zero divided by the rate constant. The flux expressed as nmol/min per mg of protein was derived from the intercept of each straight line on the ordinate.

Results

Assessment of viability of acini preparations

The morphology of typical isolated acini is shown in Fig. 1. The preparations consisted of approx. 90% acinar cells (estimated by cell counting in a haemocytometer), in the form of morphologically intact acini. A small number (approx. 5–10%) of granular, striated and intercalated ducts were also seen. At least 95% of the cells excluded Trypan Blue. Lactate dehydrogenase leakage was low (2–3% of the total cell content/h) and was unaffected by the addition of secretagogues. The ATP content of isolated acini [3.36 ± 0.48 nmol/mg of protein ($n = 4$)] was the same as that of intact submandibular tissue [3.23 ± 0.80 nmol/mg of protein ($n = 4$)]. Yields of $(7\text{--}8) \times 10^6$ acini (30–40mg of protein) were consistently obtained from 1g (wet wt.) of submandibular tissue.

Release of ^{14}C -labelled glycoproteins

Incorporation of [^{14}C]glucosamine into trichloroacetic acid/phosphotungstic acid-precipitable material was linear over at least 2h (results not shown). Increasing the time of prelabelling acini with [^{14}C]glucosamine from 90 to 180min, or the time of the post-labelling period in 1mM-glucosamine from 60 to 90min, did not affect the magnitude of subsequent secretory responses (results not shown). The 60min post-labelling period used was similar to the transit time, previously reported (Bogart, 1977), for movement of the protein core through the endoplasmic reticulum and its packaging into secretory granules of the acinar cells. The majority (80–90%) of the ^{14}C -labelled trichloroacetic acid/phosphotungstic acid-insoluble material released into the incubation medium was excluded from Bio-Gel P-200, indicating that the label was incorporated into high- M_r mucins which were secreted (McPherson *et al.*, 1983).

Adrenergic control of mucin release

The time course of ^{14}C -labelled mucin release from isolated acini and tissue fragments is shown

Fig. 1. Micrographs of isolated rat submandibular acini taken using phase contrast microscopy. Magnification 750 $\times$.

in Fig. 2. Unstimulated release was linear for at least 80 min. Mucin release was increased by approx. 3-fold in the presence of isoproterenol (10 μ M). Stimulated release was also linear, significant increases in mucin release being observed 10 min after isoproterenol addition.

The dose-dependence of isoproterenol- and noradrenaline-stimulated mucin release is shown in Fig. 3. Similar secretory responses were observed in isolated acini and tissue fragments, with maximum release at 10 μ M for both isoproterenol and noradrenaline. At submaximal agonist concentrations (0.1–1 μ M), mucin release was increased to a greater degree by isoproterenol than by noradrenaline.

Stimulation of mucin release by isoproterenol, noradrenaline, or adrenaline was significantly inhibited by the β -blocker, propranolol, but not by the α -blocker, phentolamine (Table 1), suggesting

that stimulation of β -, but not α -adrenergic receptors increases mucin release from rat submandibular acinar cells. We have also observed (results not shown) that the α -agonist phenylephrine did not stimulate mucin release in the presence of propranolol, in agreement with Quisell & Barzen (1980). It was apparent that stimulation of mucin release by mixed α - and β -agonists (adrenaline and noradrenaline) was less sensitive to inhibition by propranolol than was stimulation by the pure β -agonist, isoproterenol (Table 1).

Role of Ca^{2+} in stimulation of mucin release

Studies of the effects of removal of extracellular Ca^{2+} on mucin release from isolated acini were carried out using conditions unlikely to deplete intracellular Ca^{2+} stores before addition of secretagogue. Acini were washed once in Ca^{2+} -free medium and incubated in either Ca^{2+} (1.3 mM)-

Fig. 2. Time course of mucin release from isolated submandibular acini and tissue fragments

Acini (closed symbols) or tissue fragments (open symbols) were prelabelled as described under 'Methods', except that $1.5 \mu\text{Ci}$ of $[^{14}\text{C}]$ -glucosamine/ml was used in experiments with acini. After the 1 h post-labelling period, acini (approx. $3 \times 10^6/4\text{ml}$) or fragments (80–100 mg wet wt./2.5 ml) were incubated under the following conditions: control (●, ○); $10 \mu\text{M}$ -isoproterenol (▲, △) or $10 \mu\text{M}$ -adrenaline (■, □). Aliquots were removed from the flasks at the times shown and radioactivity incorporated into mucins was measured as described under 'Methods'. Results are means $\pm$ S.E.M. (acini) or means of duplicate flasks from a representative experiment (fragments). Control release of mucins varied from 2432 to 4117 d.p.m./80 min per mg of protein (acini) and from 4969 to 6256 d.p.m./80 min per 100 mg wet wt. (fragments). In experiments with acini, isoproterenol-stimulated mucin release gave $P < 0.02$ for difference from control, by Student's *t*-test, at all time points examined.

containing medium or Ca^{2+} -free ($+0.1 \text{ mM}$ -EGTA) medium for 60 min in the presence or absence of effectors. Isoproterenol ($10 \mu\text{M}$) increased mucin release by $334.7 \pm 25.1\%$ of control ($n = 6$) in Ca^{2+} -containing medium and by $246.1 \pm 15.7\%$ of control ($n = 6$) in Ca^{2+} -free

Fig. 3. Dose-dependence of isoproterenol- and noradrenaline-stimulated mucin release

Acini (closed symbols, solid lines) or tissue fragments (open symbols, broken lines) were prelabelled with a 1 h post-labelling period as described under 'Methods'. Mucin release was measured after 60 min incubation in medium containing various concentrations of isoproterenol (▲, △) or noradrenaline (■, □). Control release varied from 768 to 943 d.p.m./mg of protein (acini) and from 3571 to 14870 d.p.m./100 mg wet wt. (fragments). Results are means $\pm$ S.E.M. for at least four values. The curves were fitted by eye to each set of symbols.

medium ($P < 0.02$ for difference from stimulation in Ca^{2+} -containing medium). Noradrenaline ($10 \mu\text{M}$) increased mucin release by $261.9 \pm 17.9\%$ of control ($n = 4$) in Ca^{2+} -containing medium and by $154.4 \pm 5.7\%$ of control ($n = 4$) in Ca^{2+} -free medium ($P < 0.002$ for difference from stimulation in Ca^{2+} -containing medium). Removal of extracellular Ca^{2+} thus resulted in partial inhibition of stimulated mucin release, with noradrenaline stimulation being decreased to a greater extent than isoproterenol stimulation.

In order to investigate whether mobilization of intracellular Ca^{2+} is involved in stimulating mucin release, we examined the kinetics of $^{45}\text{Ca}^{2+}$ exchange in submandibular acini in response to the β -adrenergic secretagogue, isoproterenol. Fig. 4(a) shows $^{45}\text{Ca}^{2+}$ exchange in isolated acini, measured as increasing $^{45}\text{Ca}^{2+}$ content. It can be seen that isoproterenol ($10 \mu\text{M}$) increased $^{45}\text{Ca}^{2+}$ uptake, although at early time-points (2 min, 5 min, 10 min), there was no significant difference between control and stimulated uptake. After 10 min incubation, the values for $^{45}\text{Ca}^{2+}$ uptake were virtually identical ($2.46 \pm 0.07 \text{ nmol/mg}$ of protein, control, and $2.56 \pm 0.11 \text{ nmol/mg}$ of protein, stimulated; $n = 4$), with an overlap of data being observed in every experiment. Thus, the results suggest a delay of approx. 10 min before

Table 1. *Effects of α - and β -adrenergic agents on mucin release from isolated submandibular acini and tissue fragments*
Mucin release from acini or fragments during 60 min incubation was measured as described under 'Methods', under the experimental conditions shown. Control release varied from 860 to 1562 d.p.m./mg of protein (acini) and from 5300 to 10610 d.p.m./100 mg wet wt. (fragments). Numbers in parentheses represent the number of observations. * $P < 0.001$, ** $P < 0.05$ for difference from stimulation in the presence of the respective agonist in the absence of propranolol.

Experimental conditions	Mucin release (% of control)	
	Submandibular acini	Submandibular fragments
Control	100.0 $\pm$ 4.5 (8)	100.0 $\pm$ 7.5 (6)
Propranolol (30 μ M)	109.4 $\pm$ 6.3 (4)	111.0 $\pm$ 3.7 (4)
Phentolamine (30 μ M)	110.2 $\pm$ 4.9 (4)	109.7 $\pm$ 6.0 (6)
Isoproterenol (10 μ M)	466.9 $\pm$ 38.7 (10)	393.1 $\pm$ 25.1 (20)
Isoproterenol (10 μ M) + propranolol (30 μ M)	*157.6 $\pm$ 7.9 (11)	*110.5 $\pm$ 5.9 (6)
Isoproterenol (10 μ M) + phentolamine (30 μ M)	445.1 $\pm$ 33.8 (8)	452.6 $\pm$ 20.6 (6)
Noradrenaline (10 μ M)	414.9 $\pm$ 79.4 (8)	407.7 $\pm$ 26.5 (14)
Noradrenaline (10 μ M) + propranolol (30 μ M)	**220.0 $\pm$ 34.8 (8)	*194.8 $\pm$ 14.6 (6)
Noradrenaline (10 μ M) + phentolamine (30 μ M)	397.6 $\pm$ 83.1 (8)	457.1 $\pm$ 14.8 (10)
Adrenaline (10 μ M)	—	287.6 $\pm$ 27.5 (6)
Adrenaline (10 μ M) + propranolol (30 μ M)	—	**207.8 $\pm$ 12.6 (6)
Adrenaline (10 μ M) + phentolamine (30 μ M)	—	368.1 $\pm$ 49.0 (4)

Fig. 4. $^{45}\text{Ca}^{2+}$ fluxes in isolated submandibular acini

(a) $^{45}\text{Ca}^{2+}$ uptake was measured as described under 'Methods' under control conditions (●) or in the presence of 10 μ M-isoproterenol (▲). Results are means $\pm$ S.E.M. of four values; $P < 0.02$ for difference from control after 20 min by Student's *t*-test. Earlier time-points were not significantly different from control. (b). $^{45}\text{Ca}^{2+}$ efflux was measured as described under 'Methods' under control conditions (●) or in the presence of 10 μ M-isoproterenol (▲). Results are expressed as a percentage of $^{45}\text{Ca}^{2+}$ content at time zero, which varied from 2.29 to 3.84 nmol/mg of protein. Results are means $\pm$ S.E.M. of four values; $P < 0.02$ for difference from control at 30 min and 60 min time points. Earlier time points were not significantly different from control values.

Table 2. Kinetic analysis of $^{45}\text{Ca}^{2+}$ uptake into isolated submandibular acini

$^{45}\text{Ca}^{2+}$ uptake into isolated acini, in the presence or absence of isoproterenol, was measured as described under 'Methods' and the resulting curves were analysed graphically, as described. Values are mean $\pm$ S.E.M. for the number of measurements denoted in parentheses. * $P < 0.002$ for the difference from control by Student's *t*-test.

	Control	+ 10 μM -Isoproterenol
Fast phase		
Rate constant (min^{-1})	0.192 ± 0.056 (4)	0.130 ± 0.014 (4)
Pool size (nmol/mg of protein)	3.25 ± 0.79 (4)	2.81 ± 0.32 (4)
Slow phase		
Rate constant (min^{-1})	0.0068 ± 0.0005 (6)	0.0107 ± 0.0007 (4)*
Pool size (nmol/mg of protein)	9.49 ± 1.11 (6)	9.31 ± 1.21 (4)

Fig. 5. Effect of addition of isoproterenol during $^{45}\text{Ca}^{2+}$ uptake into isolated submandibular acini

●, Control uptake curve; ▲, ^{45}Ca content after addition of 10 μM -isoproterenol at the time indicated by the vertical arrow. Results are expressed as a percentage of the maximum uptake under control conditions during 150 min, which varied from 6.48 to 12.37 nmol/mg of protein. Results are means $\pm$ S.E.M. of four values; $P < 0.05$ for difference between values in the presence or absence of isoproterenol 30 min after addition of secretagogue.

increases in $^{45}\text{Ca}^{2+}$ uptake occurred in response to isoproterenol. $^{45}\text{Ca}^{2+}$ efflux from acini prelabelled for 60 min was also increased by isoproterenol (Fig. 4b), but, as for uptake, the effect was delayed. Thus, after 5 min incubation, control and stimulated rates of efflux were virtually identical [$79.0 \pm 3.2\%$ of zero-time, control, and $75.2 \pm 3.5\%$ of zero-time, +isoproterenol (10 μM); $n = 4$], and even after 15 min incubation, the difference between control ($68.9 \pm 3.7\%$ of zero time; $n = 4$) and stimulated ($60.8 \pm 3.2\%$ of zero time; $n = 4$) efflux was not significant, as determined by Student's *t* test.

The kinetic parameters derived from graphical analysis are shown in Table 2. There were two exponential phases of $^{45}\text{Ca}^{2+}$ uptake, with rate constants of the order of 0.1 min^{-1} and 0.01 min^{-1} , which are similar to those demonstrated in other mammalian cells (Borle, 1969; Claret-Berthon *et al.*, 1977; Kondo & Schulz, 1976). The faster phase of $^{45}\text{Ca}^{2+}$ uptake, which was unaffected by isoproterenol, may represent binding to the outer cell surface (Borle, 1968), whereas the slower component probably represents exchange with intracellular Ca^{2+} . Isoproterenol increased the rate constant for $^{45}\text{Ca}^{2+}$ flux into the more-slowly exchanging compartment (Table 2).

In order to investigate whether β -adrenergic stimulation of $^{45}\text{Ca}^{2+}$ exchange represented increases in net Ca^{2+} flux, the effect of adding isoproterenol after 1 h of $^{45}\text{Ca}^{2+}$ labelling, without removal of extracellular $^{45}\text{Ca}^{2+}$, was examined (Fig. 5). Isoproterenol caused a decreased $^{45}\text{Ca}^{2+}$ content which was not seen until at least 10 min after the addition. This effect was the same if isoproterenol was added after 2 h of $^{45}\text{Ca}^{2+}$ labelling (results not shown), when $^{45}\text{Ca}^{2+}$ exchange approached steady state. Thus, increased Ca^{2+} mobilization in response to isoproterenol is likely to represent a net release of Ca^{2+} from the acinar cells.

Discussion

The preparations of isolated rat submandibular acini described provide an *in vitro* preparation for studying regulation of mucin secretion, which shows similar secretory responses to those of intact tissue. The preparations are predominantly (approx. 90%) acinar cells in the normal acinar configuration. Thus, they have advantages over tissue slices (Bogart & Picarelli, 1978) and dispersed acinar-duct complexes (Quissell & Redman, 1979) in that direct actions of effectors on acinar cells can be examined. In addition, measurements of ion fluxes are facilitated since there is no large additional component from the

extracellular space. We have elsewhere reported clinical studies using these acini in which we have distinguished between the actions of control and cystic fibrosis serum in stimulating mucin release (McPherson *et al.*, 1983).

In the present study, acini preparations have been characterized by using phase-contrast microscopy, Trypan Blue exclusion, ATP content and lactate dehydrogenase leakage and have been shown to be viable in terms of these morphological and functional criteria. They also show comparable secretory responses to those of intact tissue fragments incubated *in vitro*. Thus, similar time-course, dose-dependence and degree of stimulation of mucin release by adrenergic secretagogues was observed.

Our results show that stimulation of β -, but not α -, adrenergic receptors increases mucin release from isolated acini and tissue fragments, in agreement with Bogart & Picarelli (1978). Quissell & Barzen (1980), however, reported that although β -adrenergic stimulation was essential for stimulation of mucin release, stimulation of α -adrenergic receptors could potentiate the β -effect. The reason for these differences is not clear, but could be due to the different cell preparations used. Our results, using both acini and intact tissue fragments, indicate that adrenergic control of protein secretion from the acinar cells of the rat submandibular gland, like those of the rat parotid gland (Mangos *et al.*, 1975; Kanagasuntheram & Randle, 1976; McPherson & Hales, 1978), is mediated primarily by stimulation of β -adrenergic receptors.

A role for Ca^{2+} in the stimulation of mucin release was suggested on the basis that depletion of submandibular cell Ca^{2+} (5 mM-EGTA for 1 h) resulted in inhibition of noradrenaline-stimulated mucin release (Quissell & Barzen, 1980). Removal of extracellular Ca^{2+} (see the Results section and Quissell & Barzen, 1980) resulted in only partial inhibition of isoproterenol- or noradrenaline-stimulated release, suggesting a possible involvement of intracellular Ca^{2+} store(s). Isoproterenol increased $^{45}\text{Ca}^{2+}$ exchange in rat submandibular acini, indicating increased intracellular Ca^{2+} mobilization. However, this showed a delayed (approx. 10 min) onset, whereas no corresponding delay in onset of stimulation of mucin release was apparent. In contrast, isoproterenol caused a substantial, rapid (within 2–4 min) efflux of $^{45}\text{Ca}^{2+}$ from rat parotid acinar cells (Kanagasuntheram & Randle, 1976; Butcher, 1980).

In pancreatic acini, we also observed marked increases in $^{45}\text{Ca}^{2+}$ fluxes within 1–2 min of addition of secretagogue (Dormer *et al.*, 1981), which supported the hypothesis that release of Ca^{2+} from intracellular store(s) was involved in triggering amylase secretion. Our subsequent

studies have provided direct evidence that Ca^{2+} is released from intracellular membranes (Dormer & Williams, 1981; Richardson & Dormer, 1984) and that a rise in intracellular free Ca^{2+} is the trigger for carbamylcholine stimulation of amylase release from rat pancreatic acini (Dormer, 1983, 1984).

It is apparent that secretagogue-induced increases in intracellular Ca^{2+} mobilization in mucous acinar cells are different from those in serous cells in terms of magnitude and time course, and that they are not involved in triggering mucin release. Our previous studies using cystic fibrosis serum have shown that mucin release can be stimulated in the absence of increases in $^{45}\text{Ca}^{2+}$ efflux (McPherson *et al.*, 1983). In addition, we have recently demonstrated that the bivalent cation ionophore A23187 increases intracellular free Ca^{2+} concentrations at a concentration (2 μM) which does not increase mucin release (McPherson & Dormer, 1984b), indicating that a rise in intracellular Ca^{2+} concentration is not a sufficient trigger for mucin secretion.

The significance of the increased $^{45}\text{Ca}^{2+}$ exchange mediated by β -adrenergic stimulation in rat submandibular acini is not clear. Our results suggest that this represents a small net release of Ca^{2+} from a slowly exchanging intracellular pool, which may contribute to the Ca^{2+} content of the saliva.

In conclusion, our present data do not suggest a primary role for Ca^{2+} in β -adrenergic stimulation of mucin secretion from rat submandibular acini, although Ca^{2+} may play a modulating role in maintaining stimulated rates of secretion induced by other intracellular regulator(s).

This work was supported by the Cystic Fibrosis Research Trust.

References

- Bogart, B. I. (1975) *J. Ultrastruct. Res.* **52**, 139–155
- Bogart, B. I. (1977) *Anat. Rec.* **187**, 367–382
- Bogart, B. I. & Picarelli, J. (1978) *Am. J. Physiol.* **235**, C256–C268
- Borle, A. B. (1968) *J. Cell Biol.* **36**, 567–582
- Borle, A. B. (1969) *J. Gen. Physiol.* **53**, 43–56
- Butcher, F. R. (1980) *Biochim. Biophys. Acta* **630**, 254–260
- Butcher, F. R. & Putney, J. W. (1980) *Adv. Cyclic Nucleotide Res.* **13**, 215–249
- Claret-Berthon, B., Claret, M. & Mazet, J. L. (1977) *J. Physiol. (London)* **272**, 529–552
- Dormer, R. L. (1983) *Biosci. Rep.* **3**, 233–240
- Dormer, R. L. (1984) *Biochem. Biophys. Res. Commun.* **119**, 876–883
- Dormer, R. L. & Williams, J. A. (1981) *Am. J. Physiol.* **240**, G130–G140
- Dormer, R. L., Poulsen, J. H., Licko, V. & Williams, J. A. (1981) *Am. J. Physiol.* **240**, G38–G49

- Dreisbach, R. H. (1967) *Proc. Soc. Exp. Biol. Med.* **126**, 279–281
- Kanagasuntheram, P. & Randle, P. J. (1976) *Biochem. J.* **160**, 547–564
- Kondo, S. & Schulz, I. (1976) *Biochim. Biophys. Acta* **419**, 76–92
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mangos, J. A., McSherry, N. R., Barber, T., Arvanitakis, S. N. & Wagner, V. (1975) *Am. J. Physiol.* **299**, 560–565
- Martinez, J. R. & Cassity, N. (1982) *Arch. Oral Biol.* **27**, 159–166
- Matthews, R. W. (1974) *Arch. Oral Biol.* **19**, 989–994
- McPherson, M. A. & Dormer, R. L. (1984a) *Biochem. Soc. Trans.* **12**, 652–653
- McPherson, M. A. & Dormer, R. L. (1984b) *Biochem. Soc. Trans.* **12**, 1091–1092
- McPherson, M. A. & Hales, C. N. (1978) *Biochem. J.* **176**, 855–863
- McPherson, M. A. & Schofield, J. G. (1974) *Biochem. J.* **140**, 479–485
- McPherson, M. A., Dodge, J. A. & Goodchild, M. C. (1983) *Clin. Chim. Acta* **135**, 181–188
- Quissell, D. O. & Barzen, K. A. (1980) *Am. J. Physiol.* **238**, C99–C106
- Quissell, D. O. & Redman, R. S. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 2789–2793
- Quissell, D. O., Barzen, K. A. & Lafferty, J. L. (1981) *Am. J. Physiol.* **241**, C76–C85
- Richardson, A. E. & Dormer, R. L. (1984) *Biochem. J.* **219**, 679–685
- Scott, B. L. & Pease, D. C. (1959) *Am. J. Anat.* **104**, 115–162
- Williams, J. A., Korc, M. & Dormer, R. L. (1978) *Am. J. Physiol.* **235**, E517–E524
- Yu, J. H. & Schneyer, C. A. (1983) *Proc. Soc. Exp. Biol. Med.* **173**, 386–389