Br. J. Pharmac. (1982), **77**, 405–411

THE EFFECTS OF THEOPHYLLINE AND 4-(3-BUTOXY-4-METHOXYBENZYL)-2-IMIDAZOLIDINONE (RO 20-1724) ON PROTEIN SECRETION FROM RAT PAROTID GLAND

G. AFARI, A. TENENHOUSE & C. VACHON[1]

Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada

1 The effects of two chemically distinct cyclic nucleotide phosphodiesterase (PDE) inhibitors on protein secretion from superfused rat parotid gland were studied.

2 In the presence of 1.0 mM Ca^{2+}, Ro 20-1724 (10 μM), an imidazolidinone derivative, increased the secretory response to isoprenaline 100% and the isoprenaline-dependent accumulation of adenosine cyclic 3',5'-monophosphate (cyclic AMP) 300–400%. At this concentration Ro 20-1724 alone did not cause protein secretion, accumulation of cyclic AMP or significantly inhibit PDE activity in cell-free preparations from parotid gland.

3 In the absence of added Ca^{2+} and in the presence of 1.0 mM EGTA, Ro 20-1724 inhibited the secretory response to isoprenaline 65% while increasing isoprenaline-dependent cyclic AMP accumulation 200%.

4 In the presence of Ca^{2+}, theophylline (10 mM) stimulated protein secretion but did not cause the accumulation of cyclic AMP. When combined with isoprenaline the rate of secretion was greater than the sum of the effects of the individual drugs but there was no effect of theophylline on the isoprenaline-dependent accumulation of cyclic AMP.

5 Theophylline-stimulated protein secretion is increased by omitting Ca^{2+} from the superfusion medium without any detectable change in cyclic AMP accumulation. Under these conditions Ro 20-1724 inhibits theophylline-stimulated protein secretion and the maximum rate of protein secretion in the presence of isoprenaline and theophylline is no greater than that seen with either agent alone.

6 It is concluded that the theophylline effects do not result from inhibition of PDE. It is suggested that the primary action of both drugs on parotid gland acinar cells is to alter the distribution of intracellular Ca^{2+}. Ro 20-1724 may also inhibit Ca^{2+}/calmodulin activated enzymes such as PDE.

Introduction

The methylxanthines are inhibitors of cyclic nucleotide phosphodiesterases (PDE) and as such have been used to study cellular activities believed to be mediated by adenosine cyclic 3',5'-monophosphate (cyclic AMP) (Appleman, Thompson & Russel, 1973). However, there are several reports suggesting that in certain situations, these compounds may act via different mechanisms. McNeill, Coutinho & Verma (1974) found that while theophylline increased contractility and phosphorylase activity in isolated perfused heart of guinea-pig or rat there was no associated increase in cyclic AMP concentration and Shwabe & Ebert (1972) have suggested that in fat cells the effects of theophylline are not due to inhibition of PDE. The methylxanthines are known to affect a number of other cellular processes which might account for their pharmacological action; most notable is their effect on Ca^{2+} metabolism (Nayler, 1963; Nayler & Hasker, 1966; Belleman & Scholz, 1974). In parotid gland both theophylline and caffeine have been shown to stimulate amylase secretion and to increase the secretory response of the tissue to other secretagogues (Babad, Ben-Zvi, Bdolah & Schramm, 1967; Lindsay, Ueha, Hulsey & Hansen, 1971). Although it is widely assumed that this effect is secondary to the inhibition of PDE activity and the accumulation of cyclic AMP, no attempt has been made to exclude other possible effects of these drugs.

A number of PDE inhibitors, chemically distinct from the methylxanthines have been described (Amer & Kreighbaum, 1975), among them a group of imidazolidinone derivatives developed by Hoffman-La Roche Inc. (Sheppard & Wiggan, 1970; 1971). One of these, Ro 20-1724 is reported to be from 2–1500 times more potent than theophylline; the relative potency depends on the source of the enzyme. In an effort to define more clearly the effect

[1]Present address: Departement de Dietetique, Université Laval, Québec City, Québec, Canada.

0007-1188/82/110405-07 $01.00

of inhibition of PDE activity on protein secretion from parotid gland as distinct from other actions of PDE inhibiting drugs, the effects of Ro 20-1724 and theophylline were compared.

Methods

Protein release was measured as previously described (Afari, Tenenhouse & Klein, 1977). Female Wistar rats (170–200 g) which had been fasted 18 h with free access to water were used. The rats were decapitated, the parotid glands were removed and cleaned free of adhering tissue. Protein was labelled by a 5 min exposure of the tissue to [^{3}H]-leucine (50–60 Ci/mmol) in Krebs-Ringer-Bicarbonate (KRB) buffer (composition mM: NaCl 118.5, KCl 5.9, $CaCl_2$ 1.0, $NaHCO_3$ 23.7, $MgSO_4$ 1.2, KH_2PO_4 0.6 and D-glucose 10, pH 7.4). The tissue (100 mg wet weight) was then placed in a 0.5 ml chamber and superfused at a constant rate of 0.5 ml/min with KRB containing 1 mM [^{3}H]-leucine for 2 h. After this 2 h chase superfusion the basal rate of release of ^{3}H-labelled protein from any single tissue preparation was found to vary less than ± 10%. As previously described (Afari *et al.*, 1977), under these experimental conditions all radioactivity released from the tissue spontaneously or after exposure to a secretagogue was trichloroacetic acid precipitable, therefore samples of superfusate (1.0 ml) were collected directly into scintillation vials and counted in a liquid scintillation spectrometer without further processing. After the 2 h chase superfusion, at least four 1.0 ml baseline samples were collected before the agent to be tested was added to the superfusion fluid. The tissue was superfused with the test drug for 8 min then with drug-free buffer for 60 min. One ml samples were collected throughout and radioactivity measured as described above. Results are expressed as the percentage change of protein release over basal:

$$\frac{(\text{stimulated secretion rate}) - (\text{basal secretion rate})}{(\text{basal secretion rate})} \times 100$$

Cyclic AMP accumulation was measured in parotid gland slices incubated in 2.0 ml KRB buffer at 37°C in the presence or absence of the agent to be tested. The reaction was terminated by homogenizing the entire contents of the incubation flask in trichloroacetic acid (5% final concentration) using a Polytron homogenizer (Brinkmann Instruments, Inc.). This effectively stopped the reaction within 5–10 s. The supernatant was collected, the trichloroacetic acid removed by extraction with water-saturated ether and the sample evaporated to dryness. The residue was dissolved in Tris-EDTA buffer (50 mM Tris-HCl, 4 mM EDTA, pH 7.5) and aliquots were taken for measurement of cyclic AMP by Gilman assay (Gilman, 1970).

Crude PDE was prepared as follows: rat parotid glands were homogenized in 8 volumes of 40 mM Tris HCl (pH 8.0) and the homogenate centrifuged at 1500 rev/min for 10 min. The supernatant was filtered through glass wool and the filtrate, diluted to contain 4 mg protein/ml, used in the PDE assay, employing the method described by Thompson, Brooker & Appleman (1974).

Adenylate cyclase activity was measured in a particulate fraction from homogenized parotid gland. Tissue was homogenized in 4 volumes 50 mM Tris-HCl, pH 7.5 and centrifuged at 600 *g* for 5 min. The supernatant was centrifuged at 20,000 *g* for 15 min, the pellet resuspended in 50 mM Tris-HCl, pH 7.5 and collected by centrifugation at 20,000 *g*. This wash procedure was repeated twice. The adenylate cyclase assay was done at 37°C with an incubation time of 3 min. The incubation medium (total volume 60 µl) contained 50 mM Tris-HCl, pH 7.5, 5 mM $MgSO_4$, 25 mM creatine phosphate, 450 u/ml creatine phosphokinase, 1 mM cyclic AMP, 1 mM[α-^{32}P]-ATP (40–50 ct min^{-1} pmol^{-1}). The reaction was started by the addition of enzyme preparation to yield a final protein concentration of 600 µg/ml. The reaction was stopped by addition of 140 µl of 'stopping solution' (1.4 mM cyclic AMP; 0.5 N HCl; [^{3}H]-cyclic AMP, 20,000 ct/min) and boiling for 2 min. The tubes were cooled and centrifuged at 2000 *g* for 10 min. The cyclic AMP in the supernatant was isolated on AG 50W-X4 and alumina columns and quantitated as described by Salomon, London & Rodbell (1974).

The Ro 20-1724 (4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone) was provided by Dr J.F. Meyers, Hoffman La Roche Limited, Vaudreuil, Quebec, Canada. [^{3}H]-leucine, [α-^{32}P] ATP and [^{3}H]-cyclic AMP were purchased from New England Nuclear, Lachine, Quebec; creatine phosphokinase from Boehringer Mannheim Canada Ltd, St Laurent, Quebec and the creatine phosphate from BDH Chemicals, Montreal, Quebec

Results

The effects of isoprenaline, theophylline and Ro 20-1724 on ^{3}H-labelled-protein release from rat parotid gland in the presence of extracellular Ca^{2+} is illustrated in Figure 1. There was an immediate increase in the rate of protein secretion in response to 10 µM isoprenaline to a maximum 80–100% greater than control. This maximum rate was maintained for approximately 10 min then it decreased in 45–50 min to the rate seen in unstimulated tissue. Theophylline (10 mM) stimulated ^{3}H-protein release; after a lag of 6–8 min the rate of release

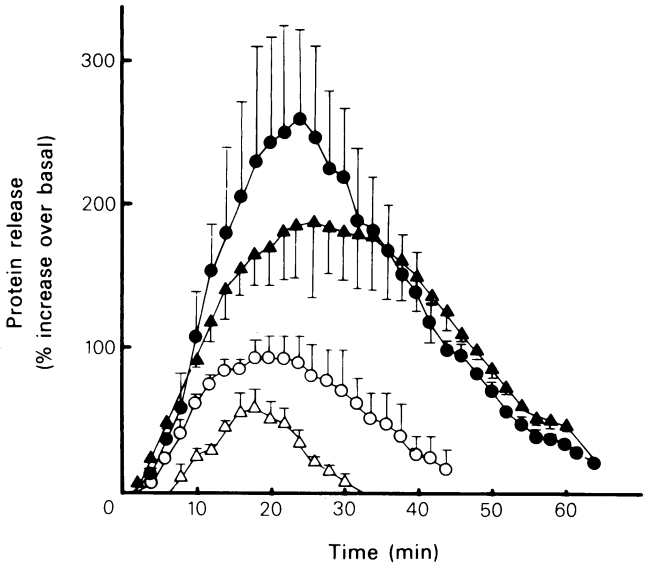

Figure 1 The effect of 10 μM isoprenaline (○), 10 mM theophylline (△), 10 μM isoprenaline plus 10 mM theophylline (●) and 10 μM isoprenaline plus 10 μM Ro 20-1724 (▲) on protein secretion from superfused rat parotid gland slices. Superfusion medium was KRB. Time zero is the time at which drug arrives at the tissue chamber. Protein release is expressed as the percentage increase over basal release. Each point is the mean of at least three experiments; vertical lines show s.e. The absolute basal rate of secretion of ^{3}H-protein ranged from 775–1025 (d min^{-1})min^{-1}. The maximum rates of secretion for each of the experimental conditions ranged from: isoprenaline, 1649–2457 (d min^{-1})min^{-1}; theophylline, 1260–1869 (d min^{-1})min^{-1}; isoprenaline + theophylline, 3499–4700 (d min^{-1})min^{-1}; isoprenaline + R020–1724, 2340–3108 (d min^{-1})min^{-1}.

increased to a maximum 60% greater than control. The combination of isoprenaline and theophylline consistently stimulated protein release to a greater extent than the sum of the effects of the individual drugs. The response to the drug combination was immediate and reached a maximum rate of release which was significantly (240–260%) greater than that seen when neither drug was present.

Ro 20-1724 (10 μM) alone did not affect the rate of ^{3}H-protein release, but when combined with isoprenaline the rate of protein release increased to 160–200% greater than control (Figure 1). The maximum rate of secretion under these conditions was significantly greater than the maximum rate of secretion seen with isoprenaline alone ($P < 0.05$). Although the secretory response to isoprenaline plus theophylline was consistently greater than the response to isoprenaline plus Ro 20-1724, this difference was not statistically significant. Ro 20-1724 had no effect on the secretory response to theophylline (results not shown).

The effect of theophylline and Ro 20-1724 on isoprenaline-stimulated cyclic AMP accumulation is illustrated in Figure 2. In untreated tissue the concentration of cyclic AMP remained constant (5.8–6.8 pmol/100 μg DNA) throughout the 6 min

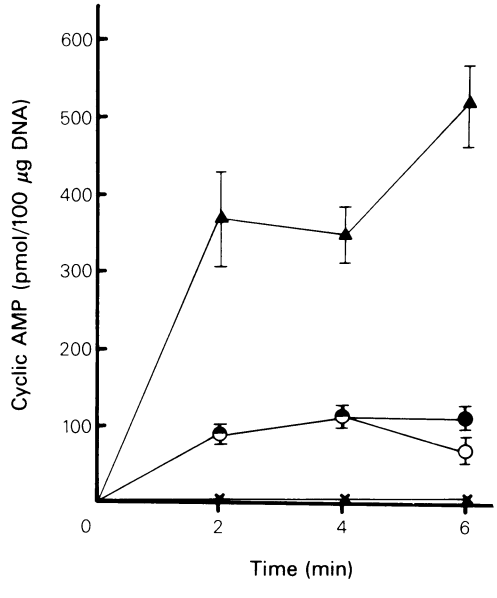

Figure 2 Cyclic AMP accumulation in rat parotid gland slices; in the absence of drug (×); 10 μM isoprenaline (○); 10 μM isoprenaline plus 10 mM theophylline (●); 10 μM isoprenaline plus 10 μM Ro 20-1724 (▲). Incubation medium was KRB.

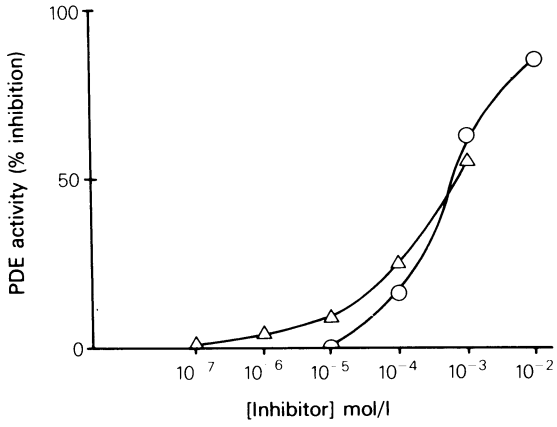

Figure 3 The effect of theophylline (○) and Ro 20-1724 (△) on cell-free parotid gland phosphodiesterase (PDE) activity. Each point is the mean of 2 or 6 separate experiments.

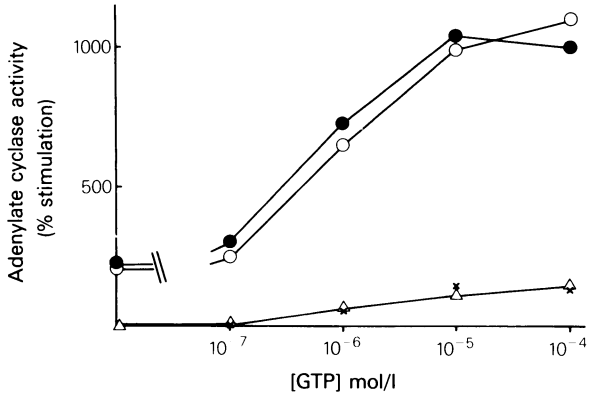

Figure 4 The effect of Ro 20-1724 on rat parotid gland adenylate cyclase activity. Basal activity (×); 10 μM Ro 20-1724 (△); 10 μM isoprenaline (○); 10 μM isoprenaline plus 10 μM Ro 20-1724 (●). Each point is the mean of 2 to 6 separate experiments.

incubation. Within 2 min after exposure to isoprenaline, the cyclic AMP concentration increased to 94.1 pmol/100 μg DNA and remained at approximately this concentration for the duration of the experiment. Theophylline itself did not increase the cyclic AMP concentration (not shown) nor did it alter the response to isoprenaline. Ro 20-1724 did not itself increase the tissue concentration of cyclic AMP but when it was combined with isoprenaline the cyclic AMP concentration increased approximately 4–5 times that seen with isoprenaline alone.

The effect of theophylline and Ro 20-1724 on the

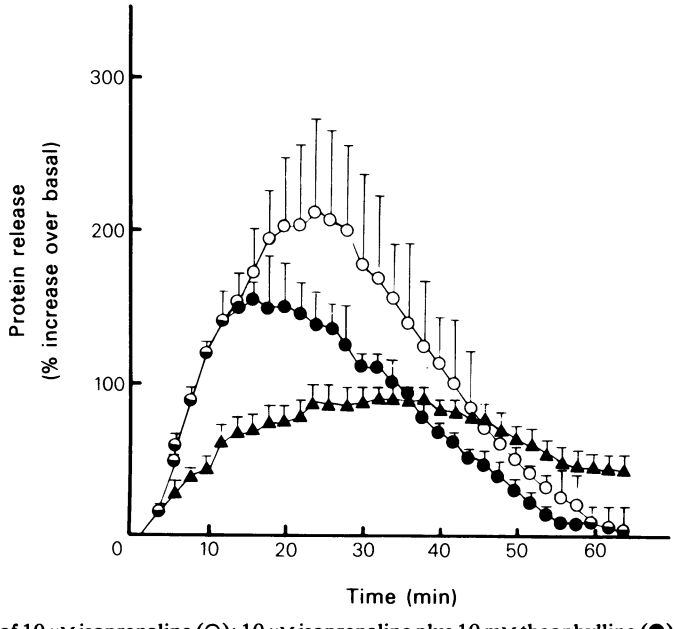

Figure 5 The effect of 10 μM isoprenaline (○); 10 μM isoprenaline plus 10 mM theophylline (●); 10 μM isoprenaline plus 10 μM Ro 20-1724 (▲) on protein secretion from rat parotid gland slices. Superfusion medium was Ca^{2+}-free KRB + 1.0 mM EGTA. Other experimental details as described in Figure 1. The absolute basal rate of secretion of ^{3}H-protein ranged from 688–1025 (d min^{-1}) min^{-1}. The maximum rates of secretion for each of the experimental conditions ranged from: isoprenaline, 2465–3840 (d min^{-1}) min^{-1}; isoprenaline + theophylline, 2019–2932 (d min^{-1}) min^{-1}; isoprenaline + R020–1724, 1426–2280 (d min^{-1}) min^{-1}.

PDE activity of cell-free preparations from rat parotid gland is shown in Figure 3. PDE activity was inhibited by theophylline at a concentration of 0.1 mM or greater; at the concentration used in the experiments described in Figures 1 and 2 (10 mM) PDE activity was inhibited 85%. Ro 20-1724 inhibited PDE activity 55% at 1.0 mM; at the concentration (10 μM) which increased isoprenaline-dependent accumulation of cyclic AMP by 300–400% it inhibited PDE activity only 9%.

It seemed unlikely that this minimal effect on PDE activity could account for the observed increase in isoprenaline-stimulated cyclic AMP accumulation caused by Ro 20-1724. The possibility that this drug directly stimulated parotid gland adenylate cyclase was therefore tested. These results are illustrated in Figure 4. Parotid gland adenylate cyclase activity was dependent on guanosine triphosphate (GTP) concentration; both basal and isoprenaline-stimulated activities were maximal at 10 μM GTP. Ro 20-1724 did not affect adenylate cyclase activity under any of the conditions tested.

The effect of theophylline and Ro 20-1724 on isoprenaline-stimulated protein release from rat parotid gland superfused with KRB containing 1.0 mM EGTA and no added Ca^{2+} is illustrated in Figure 5. The basal rate of protein secretion was unchanged by removal of extracellular Ca^{2+} (results not shown). Isoprenaline was a much more effective secretagogue in the absence of extracellular Ca^{2+} (200–220% greater than control) than in its presence (80–100% greater than control). In the absence of extracellular Ca^{2+}, Ro 20-1724 decreased the secretory response to isoprenaline approximately 65%. Under these conditions cyclic AMP accumulation after 5 min incubation with 10 μM isoprenaline was 37.9 pmol/100 μg DNA compared to 101.4 pmol/100 μg DNA in the presence of isoprenaline and 10 μM Ro 20-1724 (Figure 6). When theophylline was combined with isoprenaline the initial rate of protein release was unchanged but the maximum rate was consistently less than that seen with isoprenaline alone (Figure 5). This maximum rate was less than would have been predicted from the effects of each drug alone (Figures 5 and 7).

The effect of theophylline on protein release from rat parotid gland superfused with KRB containing 10 mM EGTA and no added Ca^{2+} is illustrated in Figure 7. Under these conditions theophylline was a more effective secretagogue than in the presence of 1 mM Ca^{2+} (compare with Figure 1). The response was immediate and the maximum rate of protein release was 180% greater than control. Ro 20-1724 itself did not affect protein release from rat parotid gland superfused with 'Ca^{2+}-free' medium but this agent did inhibit the secretory response to theophylline 80%.

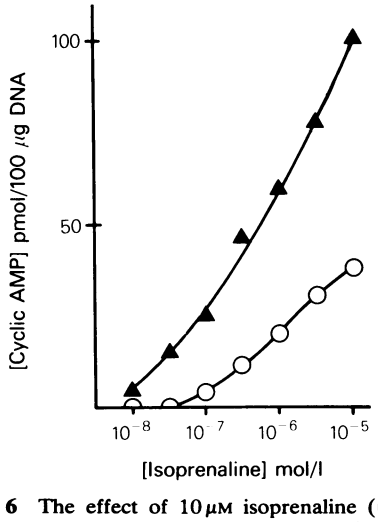

Figure 6 The effect of 10 μM isoprenaline (○) and 10 μM isoprenaline plus 10 μM Ro 20-1724 (▲) on cyclic AMP accumulation in rat parotid gland slices. Incubation medium was Ca^{2+}-free KRB + 1.0 mM EGTA and the incubation time was 5 min.

Discussion

The effects of two chemically distinct PDE inhibitors, Ro 20-1724 and theophylline, on the secretory func-

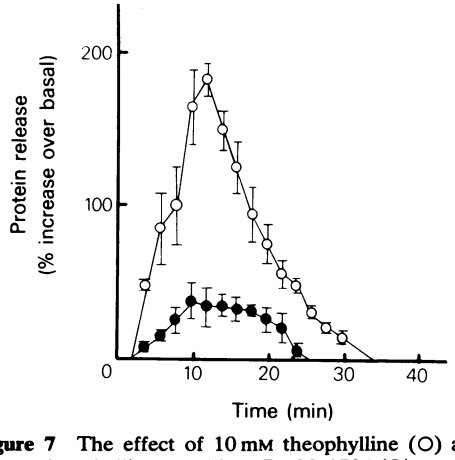

Figure 7 The effect of 10 mM theophylline (○) and 10 mM theophylline plus 10 μM Ro 20-1724 (●) on protein secretion from rat parotid gland slices. Superfusion medium was Ca^{2+}-free KRB + 1.0 mM EGTA. Other experimental conditions as described in Figure 1. The absolute basal rate of secretion of ^{3}H-protein ranged from 688–1025 (d min^{-1}) min^{-1}. The maximum rates of secretion for each of the experimental conditions ranged from: theophylline, 2165–3720 (d min^{-1}) min^{-1}; theophylline + R020–1724, 812–1165 (d min^{-1}) min^{-1}.

tion of rat parotid gland were compared. The drug concentrations were chosen such that the effect of each drug on isoprenaline-stimulated protein release under 'standard conditions' (superfused with KRB, drug added for 8 min) was approximately equal. This was found to be 10 μM Ro 20-1724 and 10 mM theophylline.

A consideration of the actions of these two drugs as illustrated by the experimental results described here suggests that they affect protein secretion from parotid gland by different mechanisms and that at least some of their actions are independent of inhibition of PDE activity. In the case of theophylline there is no evidence from our studies that this drug inhibits the enzyme when applied to tissue in concentrations which inhibit the cell-free enzyme activity 85%. In many respects theophylline and isoprenaline have very similar effects. In the presence of 1.0 mM Ca^{2+} they both stimulate protein secretion from superfused parotid gland and the secretagogue activity of both is increased when exogenous Ca^{2+} is removed. Ro 20-1724 inhibits the secretory activity of both drugs when exogenous Ca^{2+} is absent. It was also found that ouabain increased the theophylline-stimulated secretory response of parotid gland (unpublished observation) in a manner similar to its effect on isoprenaline-stimulated protein secretion (Afari *et al.*, 1977). The one major difference between the effects of isoprenaline and theophylline is that the former stimulates cyclic AMP accumulation whereas the latter does not. Also Ro 20-1724 enhances the isoprenaline-dependent secretory activity and cyclic AMP accumulation in the presence of exogenous Ca^{2+} but does not alter the response to theophylline under these conditions. The simplest mechanism of theophylline action compatible with our experimental results is that theophylline activates secretion at some step at, or distal to that activated by cyclic AMP which is believed to mediate the secretory effects of β-adrenoceptor agonists such as isoprenaline; i.e. theophylline mimics the effects of endogenously produced cyclic AMP.

The effects of Ro 20-1724 are clearly different from those of theophylline. By itself it does not cause protein secretion or cyclic AMP accumulation and its effect on secretion activated by other drugs is determined by extracellular Ca^{2+}. When Ca^{2+} is present in the superfusion medium, Ro 20-1724 appears to act as a cyclic AMP phosphodiesterase inhibitor even though at the concentration used (10 μM)it had little inhibitory effect (9%) on cell-free PDE activity. There are two immediately apparent explanations for this descrepancy. The drug may be actively taken up by parotid acinar cells so that its concentration at its site of action approaches at least 1 mM, a concentration at which cell-free PDE activity is inhibited 55%. Another possibility is that the rate of hydrolysis of cyclic AMP in the isoprenaline-stimulated parotid acinar cell is determined by a Ca^{2+}-calmodulin activated PDE (Wells & Hardman, 1977; Wolff & Brostrom, 1979) and that Ro 20-1724 prevents this activation and/or preferentially inhibits the activated enzyme. In either case the result would be to prevent the increase in PDE activity normally associated with isoprenaline activation of acinar cells which would lead to the augmented cyclic AMP accumulation and protein secretion observed. This is very similar to the mechanism by which phenothiazines are believed to inhibit cyclic nucleotide phosphodiesterases (Weiss, Fertel, Figlin & Uzunov, 1974).

A primary effect of Ro 20-1724 on Ca^{2+} metabolism might also explain the effect of this drug on parotid gland slices superfused with Ca^{2+}-free medium. The increased isoprenaline-dependent cyclic AMP accumulation caused by Ro 20-1724 under these conditions would be the result of an interference with PDE activity or activation as discussed above. In the absence of extracellular Ca^{2+} the secretory process which is believed to depend on an increased cytoplasmic [Ca^{2+}] is totally dependent on mobilization of Ca^{2+} from an intracellular source. It is possible that Ro 20-1724 interferes with this mobilization process. It is our working hypothesis that the primary action of Ro 20-1724 is to interfere with Ca^{2+} metabolism. This results in decreased capacity to mobilize Ca^{2+} from intracellular stores and to stimulate Ca^{2+}-dependent activation of enzymes one of which is PDE. This suggests that the Ro 20-1724 inhibition of theophylline-stimulated secretion which is seen when exogenous Ca^{2+} is omitted results from an interference with theophylline-dependent Ca^{2+} mobilization and therefore that the primary effect of theophylline on parotid gland is on Ca^{2+} metabolism. An effect of methylxanthines on Ca^{2+} transport has been demonstrated in other tissues and has been proposed as a major mechanism of action of these drugs in certain situations (Nayler, 1963; Nayler & Hasker, 1966; Belleman & Scholz, 1974).

It is interesting to note that in the presence of 1 mM Ca^{2+}, the combined effect of theophylline and isoprenaline on the maximum rate of protein secretion is synergistic whereas when exogenous Ca^{2+} is removed the maximum secretion rate in the presence of either drug is the same and is not increased when the drugs are combined. If our hypothesis is correct, this suggests that in the absence of exogenous Ca^{2+} both drugs rely on the same intracellular source for Ca^{2+}, and that, at the secretagogue concentrations used, the rate of mobilization of Ca^{2+} achieved with each drug alone is maximal.

References

AFARI, G., TENENHOUSE, A. & KLEIN, J. (1977). The effect of ouabain and of Ca^{2+} deprivation on isoproterenol- and DBcAMP-stimulated protein secretion from the superfused rat parotid gland. *Can. J. Physiol. Pharmac.*, **55,** 419–426.

AMER, M.S. & KREIGHBAUM, W.E. (1975). Cyclic nucleotide phosphodiesterase: Properties, activators, inhibitors, structure-activity relationships, and possible role in drug development. *J. Pharmaceut. Sci.*, **64,** 1–37.

APPLEMAN, M.M., THOMPSON, W.J. & RUSSEL, T.R. (1973). Cyclic nucleotide phosphodiesterases. *Adv. Cyclic Nucleotide Res.*, **3,** 65–98.

BABAD, H., BEN-ZVI, R., BDOLAH, A. & SCHRAMM, M. (1967). The mechanism of enzyme secretion by the cell: Effects of inducers, substrates and inhibitors of amylase secretion by rat parotid slices. *Eur. J. Biochem.*, **1,** 96–101.

BELLEMAN, P. & SCHOLZ, H. (1974). Relationship between theophylline uptake and inotropic effect in the guinea-pig heart. *Br. J. Pharmac.*, **52,** 265–274.

GILMAN, A.G. (1970). A protein binding assay for adenosine 3′,5-monophosphate. *Proc. natn. Acad. Sci. U.S.A.*, **67,** 305–312.

LINDSAY, R.H., UEHA, T., HULSEY, B.S. & HANSON, R.W. (1971). Relationship of chemically initiated enzyme secretion to metabolism in rat parotid *in vitro*. *Am. J. Physiol.*, **221,** 80–85.

McNEILL, J.H., COUTINHO, F.E. & VERMA, S.C. (1974). Lack of interaction between norepinephrine or histamine and theophylline on cardiac cyclic AMP. *Can. J. Physiol. Pharmac.*, **52,** 1095–1101.

NAYLER, W.G. (1963). Effect of caffeine on cardiac contractile activity and rediocalcium movement. *Am. J. Physiol.*, **204,** 969–974.

NAYLER, W.G. & HASKER, J.R. (1966). Effect of caffeine on calcium in subcellular fractions of cardiac muscle. *Am. J. Physiol.*, **211,** 950–954.

SALOMON, Y., LONDOS, C. & RODBELL, M. (1974). A highly sensitive adenylate cyclase assay. *Analyt. Biochem.*, **58,** 541–548.

SCHWABE, U. & EBERT, R. (1972). Different effects of lipolytic hormones and phosphodiesterase inhibitors on cyclic 3′,5′-AMP levels in isolated fat cells. *Naunyn-Schmiedebergs Arch. Pharmac.*, **274,** 287–298.

SHEPPARD, H. & WIGGAN, G. (1970). Analogues of 4-(3,4-dimethoxybenzyl)-2-imidazolidinone as potent inhibitors of rat erythrocyte adenosine cyclic 3′,5′-phosphate phosphodiesterase. *Molec. Pharmac.*, **7,** 111–115.

SHEPPARD, H. & WIGGAN, G. (1971). Different sensitivities of the phosphodiesterases (adenosine 3′,5′-cyclic phosphate 3′-phosphohydrolase) of dog cerebral cortex and erythrocytes to inhibition by synthetic agents and cold. *Biochem. Pharmac.*, **20,** 2128–2130.

THOMPSON, W.J., BROOKER, G. & APPLEMAN, M.M. (1974). Assay of cyclic nucleotide phosphodiesterases with radioactive substrates. In *Methods in Enzymology,* Vol. XXXVIII *Hormone Action, Part C, Cyclic Nucleotides*, ed. Hardman, J.G. & O'Malley, B.W. pp. 205–212. New York: Academic Press.

WEISS, B., FERTEL, R., FIGLIN, R. & UZUNOV, P. (1974). Selective alteration of the activity of multiple forms of adenosine 3′,5′-monophosphate phosphodiesterase of rat cerebrum. *Mol. Pharmac.*, **10,** 615–625.

WELLS, J.N. & HARDMAN, J.G. (1977). Cyclic nucleotide phosphodiesterases. *Adv. Cyclic Nucleotide Res.*, **8,** 119–143.

WOLFF, D.J. & BROSTROM, C.D. (1979). Properties and functions of the calcium-dependent regulator protein. *Adv. Cyclic Nucleotide Res.*, **11,** 27–88.

(Received February 8, 1982.
Revised June 10, 1982.)