THE EFFECT OF CATECHOLAMINE ANALOGUES UPON AMYLASE SECRETION FROM THE MOUSE PAROTID GLAND IN VIVO: RELATIONSHIP TO CHANGES IN CYCLIC AMP AND CYCLIC GMP LEVELS

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1. Introduction

The stimulation of amylase release from the rodent parotid gland by β-adrenergic agonists is believed to be mediated by adenosine 3′,5′-cyclic monophosphate (cyclic AMP) since they cause accumulation of cyclic AMP and their effects on both amylase release and cyclic AMP accumulation are enhanced by methylxanthines such as theophylline [1–3]. Exogenous N^6,$O^{2'}$-dibutyryl cyclic AMP (dbc-AMP) also causes amylase release [1]. However, use of the last two criteria as evidence for a role involving cyclic AMP in mediating hormone action must be done with caution since it has been reported that methylxanthines might not always act by increasing cyclic AMP levels [4]. Further, Hilz and Tarnowski have observed that dbc-AMP and cyclic AMP can have divergent biological effects [5]. Cyclic AMP has also been implicated in the release of amylase from the pancrease (see [6]).

Although cyclic AMP might mediate the actions of certain secretagogues there are indications that some of the secretagogues might act by a cyclic AMP independent mechanism. For example, the increased elevation of cyclic AMP levels in rat parotid pieces caused by epinephrine was inhibited eighty per cent by EGTA without a resulting inhibition of amylase release [7]. Also, there is considerable evidence that cyclic AMP does not mediate the action of all of the secretagogues causing amylase release from the pancreas [6,8]. Finally, cholinergic agents have no effect on cyclic AMP levels in rabbit parotid slices [9], but do increase guanosine 3′,5′-cyclic monophosphate (cyclic GMP) levels in slices of rat submaxillary glands [10] and other tissues [11]. This latter observation raises the possibility that cyclic GMP might mediate some of the actions of secretagogues which do not appear to involve cyclic AMP.

It has recently been shown that a number of catecholamine analogues stimulate DNA synthesis in mouse parotid tissue without a detectable increase in the tissue level of cyclic AMP [12]. Since in a separate series of compounds all of those compounds stimulating DNA synthesis also stimulated amylase release [13] the effect of the present series of analogues on the release of amylase and on cyclic GMP levels was investigated.

2. Materials and methods

Male Fels A or Porton mice, aged 3–4 months and weighing 26–30 g were used for all studies. Animals were kept on a 12 hr light and dark schedule and fed ad libitum until 2 hr before the experiment when food was withdrawn. Amylase activity was assayed by the method of Bernfeld, as previously described [13]. Cyclic AMP, cyclic GMP and DNA were isolated and assayed as described elsewhere [14]. The following compounds were kindly supplied free of charge: Lz-F-104 (II), R-007-XL (IV) and Sor-N-49 (V) by the Sterling–Winthrop Research Institute; isoetharine (III) by Riker Laboratories; mI 39 (VIII) and pI 39 (IX) by Boehringer Ingelheim Ltd; propranolol by ICI Ltd.; and phentolamine by Ciba Laboratories.

Isoproterenol (I) was purchased from Winthrop Laboratories; *p*-hydroxyephedrine (VII) and dichloro-

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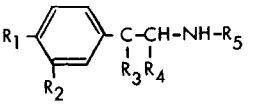
Table 1. Effect of isoproterenol analogs upon amylase and cyclic nucleotide levels in mouse parotid gland and structures of the analogs used.

Three to 6 animals were used to measure amylase levels 2 h after compound administration and groups of three animals to determine cyclic nucleotide concentrations. Cyclic AMP levels are reported in detail elsewhere (12,14), its level was increased 30-fold by isoproterenol; (-) values represent no significant difference from the control, (--) > 50% of control and (---) < 50% of control values. Amylase activity is expressed as mg maltose liberated/3 min/mg DNA and cyclic nucleotide levels as pmoles cyclic nucleotide/mg DNA.



Compound	Amount (mg)	Substituent Groups					Parotid Amylase	Cyclic AMP 15min[a]	Cyclic GMP	
		R_1	R_2	R_3	R_4	R_5			2.5min[a]	15min[a]
I	3	OH	OH	HOH	H	$CH[CH_3]_2$	70 ± 24	++++	0.53	1.29
II	4.5	OH	OH	HOH	CH_3	$CH[CH_3]_2$	70 ± 16	++		
III	3	OH	OH	HOH	CH_2CH_3	$CH[CH_3]_2$	421 ± 105	--		
IV	1.5	OH	OH	HOH	H	CH_2CH_3	1402 ± 316	-		
V	1.5	OH	OH	HOH	CH_3	CH_3	460 ± 96	-		
VI	4.5	H	OH	HOH	H	CH_2CH_3	160 ± 24	--		
VII	1.5	OH	H	HOH	CH_3	CH_3	273 ± 10	-		
VIII	3	H	OH	HOH	H	$CH[CH_3]_2$	130 ± 43	-		
IX	3	OH	H	HOH	H	$CH[CH_3]_2$	135 ± 68	--	1.19	0.52
X	3	OH	OH	O	H	$CH[CH_3]_2$	172 ± 28	-	1.46	0.62
XI	1	OH	H	H_2	H	$C[CH_3]_3$	1280 ± 170	n.d.		
XII	2	H	H	HOH	H	$CH[CH_3]_2$	81 ± 22	--	0.86	0.45
XIII	2	Cl	Cl	HOH	H	$CH[CH_3]_2$	319 ± 5	---		
Pilocarpine,	1.5						344 ± 14	-	0.42	1.22
H_2O							1191 ± 135		0.44	0.42
20% EtOH							1122 ± 149	-		
0.1 N HCl							861 ± 203	-	0.29	0.34

[a] Time after compound administration

isoproterenol (XIII) from Aldrich Chemical Co. Ltd. and S40032 (VI), S35179 (X) and S42439 (XI) from Alfred Bader Chemicals. 1-Phenyl-2-isopropyl-amino-ethanol (XII) was prepared as previously described [13]. All other drugs and fine chemicals were obtained from Sigma Chemical Co. Ltd.

Compounds were dissolved in H_2O except for VII and XI which were dissolved in 20% ethanol, XIII which was dissolved in 0.1 N HCl and reserpine which was dissolved in acid 20% acetone. All were injected intraperitoneally in a volume of 0.2 ml.

3. Results and discussion

The effect of isoproterenol and a number of its analogues upon amylase release, cyclic AMP and cyclic GMP levels in the mouse parotid gland are shown in table 1. Release of amylase is indicated by the loss of its activity from the gland. Cyclic AMP levels were measured at 15 min after compound administration as this is at the peak of cyclic AMP concentration after isoproterenol injection [12]. The analogues without a stimulatory effect at 15 min were also without effect

at 2.5 min. Compound XII and pilocarpine have no effect over a wide time course [14].

Increasing the size of the amino substituent group (R_5) had little effect upon the ability to deplete amylase or increase cyclic AMP levels and such analogues and therefore omitted from table 1. In contrast, reducing the size of this substitutuent, as in IV, immediately leads to a complete loss of effect on enzyme release and cyclic AMP levels. The addition of a substituent (R_4) on the α-carbon atom has no effect on amylase release if a methyl group (II) and is moderately inhibitory if an ethyl group (III), while these substituents halve and abolish the stimulation in cyclic AMP levels respectively. The removal of one or both of the aromatic ring hydroxyl groups has no effect on the ability to induce amylase release (VIII, IX, XII) and such a modification actually reactivates compounds with smaller R_5 substituents (VI, VII). Similarly, replacement of the catechol hydroxyl groups with chlorine atoms only partially inactivates (XIII) with respect to amylase release. Removal of the hydroxyl substituent on the β-carbon atom is also tolerated (X) but removal of this group plus one of the ring hydroxyls leads to complete inactivation (XI). None of the compounds VI to XIII has any stimulatory effect on cyclic AMP levels. Pilocarpine, a compound structurally unrelated to isoproterenol, causes a sympathetic system-mediated secretion by an indirect action via the superior cervical ganglion [15]. It also has no effect on cyclic AMP levels in agreement with Guidotti et al. [16].

Although a redistribution of cyclic AMP resulting in a local increase in concentration cannot be ruled out, this would seem to be made less likely by the fact that compounds VI, IX, XII & XIII actually decrease the concentration of cyclic AMP in the parotid and analogues with no effect on cyclic AMP in vivo do not activate adenylate cyclase in vitro [12].

An alternative mechanism would therefore seem to be operating to mediate the stimulation of secretion by many, and possibly all, of the compounds tested. An increase in the intracellular concentration of cyclic GMP could be such a mechanism. Cholinergic agents increase the concentration of cyclic GMP in many tissues, including the submaxillary gland [10]. However in all systems so far tested isoproterenol has been found to have no effect on or actually to inhibit increases in, cyclic GMP levels [11]. By contrast, isoproterenol produces a 3-fold increase in the level of cyclic GMP in the parotid. Compounds IX, X, XII as well as pilocarpine also increase the cyclic GMP concentration (table 1). To our knowledge this is the first report of a possible role for cyclic GMP in amylase release from the parotid gland. Butcher (unpublished observations) has shown that α-adrenergic and cholinergic agents increase the cyclic GMP level in rat parotid slices. The composition of the saliva secreted after β-adrenergic stimulation is quite distinct from that secreted after either cholinergic or α-adrenergic stimulation. The former is very concentrated with a high ratio of amylase to saliva volume while the latter stimulation leads to a low ratio of amylase concentration to saliva volume. In the submaxillary gland the duct cells are responsible for the major part of the water transfer which is characteristic of cholinergic and α-adrenergic secretion [17]. The acinar and ductal mechanisms for water transfer are under separate control [17]. The acinar cells of the parotid are innervated through close contacts with both adrenergic and cholinergic nerves suggesting a dual functional innervation [18]. In an attempt to separate out different stimulatory mechanisms the effects of the α-adrenergic blocking agent, phentolamine, the β-adrenergic antagonist, propranolol; the cholinergic antagonist, atropine; and reserpine which depletes the endogenous catecholamine stores on the secretion of amylase from the parotid was studied (table 2). Depletion of amylase by compounds I and IX was unaffected by atropine and phentolamine and reserpine had only a small effect but propranolol inhibited strongly suggesting that these compounds are directly acting β-agonists. Secretion of amylase induced by V and X was completely inhibited by both reserpine and propranolol, suggesting the possible mediation by endogenous catecholamine release resulting in β-adrenergic activation. Secretion caused by XII and pilocarpine was largely but not completely inhibited by reserpine and propranolol and there was a partial inhibition by atropine suggesting a parasympathetic component as is known to exist with pilocarpine.

These results suggest that differing catecholamine analogues may have different modes of action and that cyclic GMP may be involved in mediating some of their actions while cyclic AMP is not involved. However the effects are extremely complex and are no doubt further complicated by the fact that calcium ions are also known to play a role in controlling secretion [7,19].

Table 2
Effect of inhibitors on the depletion of parotid amylase by catecholamine analogues

Agonist	Amount (mg)	Antagonist				
		None	Phentolamine 2	Reserpine 0.1	Atropine 1	Propranolol 2
		mg maltose liberated/3 min/mg DNA				
H_2O	–	1095	560	1194	1433	1015
I	1	73	24	332	64	806
V	1	483	206	955	751	1203
IX	1	117	29	298	201	912
X	1	274	49	1281	504	1261
XII	2	157	243	761	615	937
pilocarpine	1	535	69	962	921	864

Inhibitors were administered 10 min prior to the analogues, except for reserpine which was given 24 hr previously. Other conditions were as described under Materials and methods and in table 1.

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References

[1] Bdolah, A. and Schramm, M. (1965) Biochem. Biophys. Res. Commun. 18, 452–454.
[2] Babad, H., Ben-Zvi, R., Bdolah, A. and Schramm, M. (1967) Eur. J. Biochem. 1, 96–101.
[3] Malamud, D. (1972) Biochim. Biophys. Acta, 279, 373–376.
[4] Schwabe, U. and Ebert, R. (1972) Archiv. fur Pharmacol. 274, 287–298.
[5] Hilz, H. and Tarnowski, W. (1970). Biochem. Biophys. Res. Commun. 40, 973–981.
[6] Bauduin, H., Rochus, L., Vincent, D. and Dumont, J. E. (1971). Biochim. Biophys. Acta, 252, 171–183.
[7] Harfield, B. and Tenenhouse, A. (1973). Can. J. Physiol. Pharmacol. 51, 997–1001.
[8] Heisler, S., Fast, D. and Tenenhouse, A. (1972). Biochim. Biophys. Acta, 279, 561–572.
[9] Monnard, P. and Schorderet, M. (1973). Eur. J. Pharmacol. 23, 306–310.
[10] Schultz, G., Hardman, J. G., Schultz, K., Baird, C. E. and Sutherland, E. W. (1973). Proc. Natl. Acad. Sci. US. 70, 3889–3893.
[11] Goldberg, N. D., O'Dea, R. F. and Haddox, M. K. (1973). Adv. in Cyclic Nucleotide Res. 3, 155–224.
[12] Durham, J. P., Baserga, R. and Butcher, F. R. (1974) in: Control of proliferation in animal cells, (Clarkson, B. and Baserga, R., eds.), Cold Spring Harbor Laboratory, New York, p. 595–607.
[13] Kirby, K. C., Swern, D. and Baserga, R. (1969). Mol. Pharmacol. 5, 572–579.
[14] Durham, J. P., Baserga, R. and Butcher, F. R. Biochim. Biophys. Acta, in press.
[15] Schneyer, C. A. and Hall, H. D. (1966). Proc. Soc. Exptl. Biol. Med. 121, 96–100.
[16] Guidotti, A., Weiss, B. and Costa, E. (1972). Mol. Pharmacol. 8, 521–530.
[17] Martinez, J. R. and Martinez, A. M. (1972). Eur. J. Pharmacol. 18, 375–385.
[18] Hand, A. R. (1972). Anat. Rec. 173, 313–339.
[19] Rasmussen, H. (1972). C.R.C. Critical Reviews in Biochem. 1, 95–148.