ELSEVIER

European Journal of Pharmacology 322 (1997) 21–25

ejp

Effects of K^+ channel blockers and K^+ ionophore on isoprenaline-induced secretion of amylase from rat parotid acini

Takafumi Murayama [b], Yoshihiro Miwa [a], Sadaaki Maeda [a], Ichijiro Morisaki [b], Kihachi Saito [a,*]

[a] *Department of Pharmacology, Osaka University, Faculty of Dentistry, Yamadaoka 1-8, Suita, Osaka 565, Japan*
[b] *Clinical Department of Dentistry for the Disabled, Osaka University, Faculty of Dentistry, Yamadaoka 1-8, Suita, Osaka 565, Japan*

Received 11 July 1996; revised 16 December 1996; accepted 17 December 1996

Abstract

The involvement of K^+ channels in regulating secretion of amylase from isolated rat parotid acini was studied in conjunction with β-adrenoceptor function. It was observed that increasing the concentration of extracellular K^+ or adding K^+ channel blockers enhanced the secretion of amylase. Among several K^+ channel blockers, tetraethylammonium, apamin and charybdotoxin were effective to enhance secretion by 48, 69 and 84%, respectively. Glibenclamide was without effect. A low concentration of isoprenaline (10^{-7} M) enhanced secretion by 154% and its simultaneous application with tetraethylammonium gave a synergistic effect, producing 371% stimulation. Combination of tetraethylammonium and a low concentration of carbachol (10^{-6} M) did not give the synergistic effect. Isoprenaline at the concentration of 10^{-6} M enhanced secretion by 313% and this was reduced to 116% by 10^{-5} M valinomycin, a K^+ ionophore. Valinomycin was without effect on carbachol (10^{-5} M)-induced secretion. Somatostatin (10^{-5} M) and morphine (10^{-4} M) also reduced isoprenaline-induced secretion of amylase. These results suggested the regulation of Ca^{2+}-activated K^+ channels by isoprenaline in amylase secretory processes in parotid acini.

Keywords: Amylase secretion; K^+ channel; Parotid acini; Isoprenaline

1. Introduction

Both adrenergic and cholinergic nerves innervate the parotid gland and regulate secretion of saliva through a distinct process. Activation of β-adrenoceptors induces secretion of saliva, resulting from an increase in the formation of cyclic AMP (Baum et al., 1981). Stimulation of muscarinic receptors causes secretion through the formation of inositol 1,4,5-triphosphate (IP_3) followed by the increase of intracellular Ca^{2+} (Tojyo et al., 1992). In addition, activation of β-adrenoceptors induces a small amount of mucous secretion while that caused by muscarinic stimulation is profuse and rich in K^+.

It is well known that Ca^{2+}-activated K^+ channels (K_{Ca}) are located on the basolateral plasma membrane of parotid acini. The channels are activated by the increase of intracellular Ca^{2+} and cause the efflux of K^+. Co-transport of Cl^- with Na^+ and K^+ into the cell across the submucosal surface follows and Cl^- is then secreted from the apical surface after transepithelial Cl^- transport. Thus, salt exit and accompanying fluid secretion are induced through processes involving K_{Ca} channel activity without osmotic perturbation (Petersen and Maruyama, 1984; Putney, 1986; McCann and Welsh, 1990). Modulation of K_{Ca} channels by cyclic AMP has been suggested in other systems (Levitan, 1994). K_{Ca} may be involved in regulating the secretion of amylase in parotid gland subsequent to β-adrenoceptor stimulation. In the present study, correlation of β-adrenoceptor stimulation and K^+ channel activity in secretory processes was examined in rat parotid acinar cells.

2. Materials and methods

2.1. Cell preparation

Male Sprague-Dawley rats (180–220 g) were housed at least a week before use under a 12-h light-dark cycle. Food and water were provided ad libitum. The rats were lightly anesthetized with ether and killed by decapitation. Parotid

* Corresponding author. Tel.: (81-6) 879-2910; Fax: (81-6) 879-2914.

0014-2999/97/$17.00
PII S0014-2999(96)00990-9

glands were ioslated, cut into small pieces and digested in 6 ml Hanks solution (composition: NaCl 136 mM, KCl 5.4 mM, Na_2HPO_4 0.34 mM, KH_2PO_4 0.44 mM, $MgSO_4$ 0.81 mM, $NaHCO_3$ 4.2 mM, glucose 5.6 mM, $CaCl_2$ 1.3 mM, Hepes 20 mM pH 7.4) containing collagenase (114 unit/ml, Worthington) and hyaluronidase (0.25 mg/ml, Sigma) at 37°C for 45–60 min (Tojyo et al., 1987; Miwa et al., 1996). Parotid acinar cells were separated from cell debris by passage through nylon mesh and were centrifuged at 1000 rpm for 5 min. The resultant pellet was resuspended in Hanks solution containing 0.1% bovine serum albumin and centrifuged at 1000 rpm for 5 min. This was repeated three times. Cells obtained from a single rat were resuspended in the same solution to give a concentration of 45–80 μg protein/ml and used in experiments.

2.2. Measurement of secretion of amylase from parotid acinar cells

Aliquots (500 μl) of cell suspension were incubated in triplicate at 37°C for 30 min in the absence and presence of drugs. The reactions were terminated by passing the incubation mixture through filters (Millex, Millipore). The amount of amylase secreted into filtrates was determined by measuring the formation of glucose from amylose with an assay kit (Amylase B-test, Wako). Triplicate measurements were averaged and the data were expressed as percentage of secretion, taking that observed without drugs as 100% (control).

3. Results

The amounts of amylase contained in isolated parotid acini and secreted during a 30-min incubation differed depending on the preparations. The isolated parotid acini employed in the present study contained $10.0–55.5 \times 10^5$ (av. 40.6×10^5) units amylase/mg cell protein and secreted $1.0–6.7 \times 10^5$ (av. 2.9×10^5) unit amylase/mg cell protein. The results were expressed as % of control secretion.

In order to determine whether a change in K^+ equilibrium potential or blockade of K^+ channels affects secretion of amylase, the effects of increasing concentrations of K^+ or K^+ channel blockers were examined. An increase in extracellular K^+ concentration to 50 mM enhanced secretion by 50% (Table 1). It was observed that tetraethylammonium enhanced secretion. Since tetraethylammonium is a non-selective blocker of K^+ channels, apamin, charybdotoxin and glibenclamide were used to block K_{Ca} and ATP-dependent K^+ channels. Secretion was enhanced by apamin and charybdotoxin. Glibenclamide was without effect on secretion.

Although the effect of tetraethylammonium to induce secretion of amylase was significant, the extent of the enhancement was small. We included isoprenaline or carbachol simultaneously to see whether the effect of tetraethylammonium is enhanced by isoprenaline or carbachol. Isoprenaline gave dose-dependent effects, enhancing secretion to 254 ± 83, 245 ± 43, 397 ± 49 and 381 ± 90% at the concentration of 10^{-7} M, 5×10^{-7}, 10^{-6} and 10^{-5}

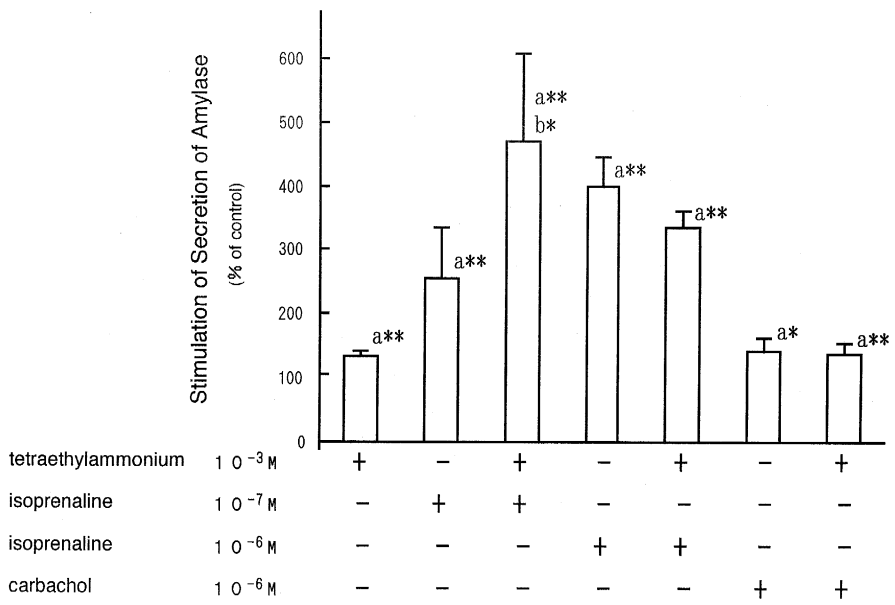

Fig. 1. Effect of tetraethylammonium on isoprenaline- and carbachol-induced secretion of amylase. Values are means ± S.D. of 5 experiments. [a]Significantly different from control. [b]Significantly different from isoprenaline-induced secretion. ($^{**}P < 0.01$, $^{*}P < 0.05$ as determined by Student's *t*-test).

Table 1
Effects of an increase in extracellular K^+ or K^+ channel blockade on secretion of amylase

Compound	Dose	Secretion of amylase (% of control)
K^+	10 mM	120 ± 8
	20 mM	124 ± 18
	50 mM	150 ± 11 [a]
Tetraethylammonium	10^{-3} M	148 ± 22 [a]
Apamin	10^{-6} M	169 ± 38 [a]
Charybdotoxin	2×10^{-6} M	184 ± 28 [a]
Glibenclamide	10^{-5} M	107 ± 17

Values are means ± S.D. of 4 experiments. [a] Significantly different from control (determined by Student's *t* -test).

Table 2
Effects of somatostatin and opioids on isoprenaline-induced secretion of amylase

Compound	Dose	Secretion of amylase (% of control)
Isoprenaline	10^{-6} M	413 ± 136 [a]
Somatostatin	10^{-5} M	113 ± 23
Morphine	10^{-4} M	107 ± 20
Isoprenaline		
+ somatostatin		228 ± 73 [b]
+ morphine		240 ± 77 [b]
Tetraethylammonium	10^{-3} M	143 ± 19 [a]
+ morphine	10^{-4} M	103 ± 5 [c]
+ DAGO	10^{-5} M	107 ± 10 [c]
+ DPDPE	10^{-5} M	126 ± 13
+ ethylketocyclazocine	10^{-5} M	145 ± 20

Values are means ± S.D. of 4–5 experiments. [a] Significantly different from control. [b] Significantly different from isoprenaline-induced secretion. [c] Significantly different from tetraethylammonium-induced secretion (determined by Student's *t*-test). DAGO: Tyr-D-Ala-Gly-MePhe-Gly-ol; DPDPE: D-Pen-D-Pen-Enkephalin.

M, respectively ($n = 4$). When 10^{-3} M tetraethylammonium was included simultaneously with 10^{-7} M isoprenaline, the enhancement of secretion was more than the sum of the individual effects (Fig. 1). Tetraethylammonium with 10^{-6} M isoprenaline, at which concentration of isoprenaline an almost maximal effect was observed, did not give a potentiating effect. Carbachol at the concentration of 10^{-6} M stimulated secretion to 141%. Tetraethylammonium was without effect on carbachol-induced secretion of amylase.

The effect of valinomycin, a K^+ ionophore, on isoprenaline- and carbachol-induced secretion of amylase was examined (Fig. 2). Valinomycin (10^{-5} M) alone enhanced secretion. The secretion induced by isoprenaline (10^{-6} M) was reduced by valinomycin while that induced by carbachol was not reduced. Instead, the effects of valinomycin and carbachol were additive.

It has been shown that somatostatin and morphine reduce release and/or secretion by enhancing K^+ conductance (North et al., 1987; White et al., 1991). As shown in Table 2, both somatostatin and morphine reduced the isoprenaline-induced secretion of amylase. The effect of morphine on tetraethylammonium-induced secretion was also examined to demonstrate the involvement of K^+ channels in the inhibitory effect of morphine. Determination of the types of opioid receptors involved in the regulation of amylase secretion was also attempted. As shown in Table 2, morphine diminished the tetraethylammonium-induced secretion of amylase. Among opioid receptor agonists, Tyr-D-Ala-Gly-MePhe-Gly-ol, an μ-opioid receptor agonist, was effective.

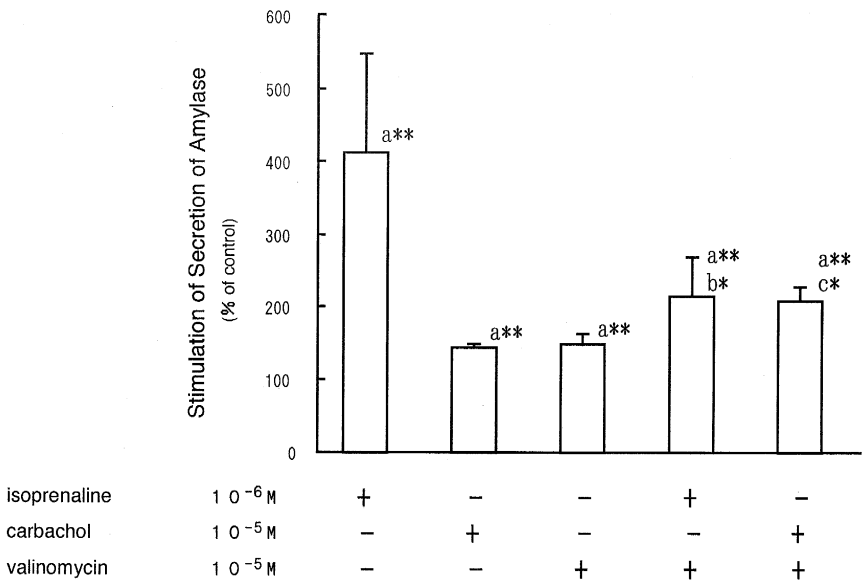

Fig. 2. Effect of valinomycin on isoprenaline- and carbachol-induced secretion of amylase. Values are means ± S.D. of 4–5 experiments. [a]Significantly different from control. [b]Significantly different from isoprenaline-induced secretion. [c]Significantly differernt from carbachol-induced secretion. ($^{**}P < 0.01$, $^{*}P < 0.05$ as determined by Student's *t*-test).

4. Discussion

Stimulation of muscarinic receptors or α-adrenoceptors causes an increase of IP_3 formation followed by an increase of intracellular Ca^{2+} in parotid acinar cells (Tojyo et al., 1992). Subsequent activation of K_{Ca} channels induces the efflux of K^+ and uptake of Na^+, K^+ and Cl^-. Cl^- is then transported to apical sites and secreted into the lumen by Ca^{2+}-dependent Cl^- channels. Thus, a regulatory role of basolateral membrane K_{Ca} channels on transepithelial Cl^- secretion has been suggested (Petersen and Maruyama, 1984; Putney, 1986; McCann and Welsh, 1990). Regulation of K_{Ca} channels by cyclic AMP also has been suggested (Baum et al., 1981). Accordingly, we examined the relation of β-adrenoceptor stimulation, K_{Ca} channel activity and amylase secretion in parotid acinar cells.

Blocking K^+ channels extends action potentials, depolarizes membranes and induces secretion. The increase in the secretion of amylase following an increase in extracellular K^+ concentration and application of tetraethylammonium indicated voltage dependence and involvement of K^+ channels in the regulation of secretion. Enhancement of secretion by apamin and charybdotoxin but not by glibenclamide indicated involvement of K_{Ca} channels. Three kinds of K_{Ca} channels, small (SK_{Ca}), large (BK_{Ca}) conductance and others, has been proposed (Sah, 1996). BK_{Ca} have been shown on the basolateral membrane of parotid acinar cells by patch-clamp experiments (Maruyama et al., 1983). BK_{Ca} are blocked by charybdotoxin while SK_{Ca} are blocked by apamin. In the present study, charybdotoxin and apamin enhanced secretion, suggesting the involvement both in the secretory process.

However, the enhancement of secretion by K^+ channel blockade was very slight compared with that induced by isoprenaline. This could be explained by the low conduction of K^+ when cells are not stimulated. Therefore, we applied isoprenaline or carbachol simultaneously with tetraethylammonium. Under these circumstances, when cells are stimulated, blockade of K^+ channels might give a synergistic effect by inhibiting repolarization. This was observed for the effect of isoprenaline but not for carbachol.

Valinomycin increases membrane K^+ permeability and would inhibit secretion and/or release. As expected, secretion of prolactin from anterior pituitary cells was inhibited by valinomycin (Wang et al., 1994). However, stimulation of secretion of renin from renal cortical slices by valinomycin was also reported (Park et al., 1991). The latter effect was explained by osmotic swelling and exocytosis caused by the change of K^+ permeability across the secretory granule membrane. This effect was not confined to K^+ ionophore and was also observed with other monovalent cation ionophores. Stimulation of amylase secretion by valinomycin might be related with this observation. Despite the stimulating effect of valinomycin on amylase secretion, it reduced isoprenaline-induced secretion.This might be related with a change in K^+ conductance like that produced by somatostatin (discussed below). The lack of effect of valinomycin on carbachol-induced secretion also discriminated between isoprenaline and carbachol in secretory processes.

It has been reported that somatostatin and morphine enhance K^+ conductance, thereby inhibiting release and/or secretion (North et al., 1987; White et al., 1991). The reduction of isoprenaline-induced amylase secretion by both somatostatin and morphine further supported an involvement of K^+ channels in the enhancement of secretion. The K^+ channels associated with the effect of somatostatin have been suggested to be BK_{Ca} channels in pituitary tumour cells (White et al., 1991). This is compatible with the present observation that BK_{Ca} are involved in regulating amylase secretion. K^+ channels involved in the effect of morphine in the central nervous system have been considered to be inwardly rectifying (North et al., 1987) or ATP-dependent (Welch and Dunlow, 1993) channels. As discussed above, the lack of effect of glibenclamide on amylase secretion indicates an absence of ATP-dependent, and possibly also of inwardly rectifying, K^+ channels. Thus, morphine might affect BK_{Ca} in parotid acinar cells in the same way as does somatostatin.

Acknowledgements

This work was supported by a research grant from the Scientific Research Fund of the Ministry of Education, Science and Culture of Japan.

References

Baum, B.J., J.M. Freiberg, H. Ito, G.S. Roth and C.R. Filburn, 1981, β-Adrenergic regulation of protein phosphorylation and its relationship to exocrine secretion in dispersed rat parotid gland acinar cells, J. Biol. Chem. 256, 9731.

Levitan, I.B., 1994, Modulation of ion channels by protein phosphorylation and dephosphorylation, Annu. Rev. Physiol. 56, 193.

Maruyama, Y., D.V. Gallacher and O.H. Petersen, 1983, Voltage and Ca^{2+}-activated K^+ channel in baso-lateal acinar cell membranes of mammalian salivary glands, Nature 302, 827.

McCann, J.D. and M.J. Welsh, 1990, Basolateral membrane potassium channels in secretory epithelia, in: Potassium Channels: Structure, Classification, Function and Therapeutic Potential, ed. N.S. Cook (Ellis Horwood, Englewood Cliffs, NJ) p. 326.

Miwa, Y., M. Saeki, A. Yamaji, S. Maeda and K. Saito, 1996, Effect of morphine on secretion of amylase from isolated parotid acini, Life Sci. (in press).

North, R.A., J.T. Williams, A. Surprenant and M.J. Christie, 1987, μ and δ receptors belong to a family of receptors that are coupled to potassium channels, Proc. Natl. Acad. Sci. USA 84, 5487.

Park, C.S., T.W. Honeyman, S.K. Ha, H.K. Choi, C.L. Chung and C.D. Hong, 1991, Mechanisms of ionophore-induced stimulation of renin secretion with special reference to a chemiosmotic hypothesis, J. Pharmacol. Exp. Ther. 259, 211.

Petersen, O.H. and Y. Maruyama, 1984, Calcium-activated potassium channels and their role in secretion, Nature 307, 693.

Putney, J.W., 1986, Identification of cellular activation mechanisms associated with salivary secretion, Annu. Rev. Physiol. 48, 75.

Sah, P., 1996, Ca^{2+}-activated K^+ currents in neurons; types, physioloical roles and modulation, Trends Pharmacol. Sci. 19, 150.

Tojyo, Y., M. Uchida and Y. Matsumoto, 1987, Inhibitory effects of calmodulin antagonists on isoproterenol- and dibutyryl cyclic AMP-stimulated amylase release from rat parotid acinar cells, Jpn. J. Pharmacol. 45, 487.

Tojyo, Y., S. Matsui, A. Tanimura and Y. Matsumoto, 1992, Relationship between cytosolic Ca^{2+} concentration and amylase release in rat parotid acinar cells following muscarinic stimulation, Biochim. Biophys. Acta 1134, 278.

Wang, X., T. Inukai, M.A. Greer and S.E. Greer, 1994, Rat Prl and TSH secretion are regulated differently by K^+-channel blokers, Am. J. Physiol. 266, E39.

Welch, S.P. and L.D. Dunlow, 1993, Antinociceptive activity of intrathecally administered potassium channel openers and opioid agonists: A common mechanism of action?, J. Pharmacol. Exp. Ther. 267, 390.

White, R.E., A. Schonbrunn and D.L. Armstrong, 1991, Somatostatin stimulates Ca^{2+}-activated K^+ channels through protein dephosphorylation, Nature 351, 570.