SODIUM REQUIREMENT IN SECRETORY PROCESSES REGULATED THROUGH MUSCARINIC RECEPTORS IN RAT PAROTID GLANDS

Its effect on amylase secretion and phosphatidylinositol labelling

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1. Introduction

In rat parotid glands, the cholinergic stimulation through the muscarinic receptor enhances amylase secretion and potassium efflux [1–4] Both responses require calcium in the extracellular medium [1,2,4] The cholinergic effects are mimicked by ionophore A 23187 which introduces Ca^{2+} into the acinar cell [5,6] and inhibited by antagonists of Ca^{2+} movements like lanthanum [2,7] The cholinergic agonist stimulates $^{45}Ca^{2+}$ uptake in rat parotid glands and this effect is also abolished by lanthanum [7,8] Therefore the primary event of cholinergic stimulation could be Ca^{2+} entry into the cell On the other hand the muscarinic stimulation also produces an increased turnover of the phosphoinositol group in phosphatidylinositol [7,9] However this last effect is independent of exogenous Ca^{2+} and is not mimicked by ionophore A 23187 [5,10] By using lanthanum we have demonstrated a relationship between the phospholipidic effect induced by cholinergic agonist (independent of calcium), the Ca^{2+} uptake and the secretory processes (dependent on extracellular Ca^{2+}) [7]

As opposed to the role of Ca^{2+}, the role of Na^+ is not well known Cholinergic agonists have been shown to enhance Na^+ uptake in rat parotid glands [11] However this latter effect is also dependent on a Ca^{2+} entry

This work was undertaken to study the importance of the variations of Na^+ concentration gradient between extra- and intracellular medium in relation to the phosphatidylinositol turnover and amylase secretion induced by muscarinic cholinergic stimulation

2 Materials and methods

myo-[2-^{3}H]Inositol (3 5 Ci/mmol) was purchased from Radiochemical Centre (Amersham) Carbamylcholine was obtained from Mann Research, New York, Tes from Serva Feinbiochemica, (Heidelberg), L-isoproterenol-d-bitartrate from Sigma (Saint-Louis, MO) Incubations were performed in Tes–Ringer buffer (5 mM Tes, 1 35 mM NaCl, 4 75 mM KCl, 1 2 mM $MgCl_2$, 0 55 mM glucose pH 7 0) equilibrated with 100% O_2 When solutions at low Na^+ concentrations were prepared, osmolarity was maintained with pH 7 0 Tris buffer

For amylase experiments, fragments of parotid glands (100–150 mg) were first washed 3-fold with Tes–Ringer buffer of the same ionic composition as subsequent incubations The fragments of gland were incubated at 37°C for 40 min in Tes–Ringer buffer at different Na^+ concentrations in the presence or absence of Ca^{2+} The cholinergic agonist was added at the onset of incubation At the end of the incubation, glands were homogenized in 0 02 M phosphate, 0 15 M NaCl buffer and centrifuged at 40 000 × *g* for 30 min Amylase in supernatant after homogenization and incubation medium was assayed according to [12] Amylase release was expressed as the amount of

amylase present in the incubation medium as a % of the sum of amylase in supernatant of homogenized glands and in incubation medium.

For phospholipid labelling, parotid glands were washed as above and incubated at 37°C for 40 min with 5 μCi or 10 μCi *myo*-[2-^{3}H]inositol in 7 ml Tes–Ringer buffer at various Na^+ concentrations. At the end of incubation, parotid fragments were blotted, weighed and homogenized in 4 ml cold trichloroacetic acid (TCA) 10%. The TCA homogenate was centrifuged at 4000 × *g* for 20 min. The pellet was washed 2-fold with 2 ml TCA 10%. The supernatants contain mainly the free *myo*-[2-^{3}H]inositol (TCA-soluble fraction). Then phospholipids from the pellet were extracted with 2 ml chloroform–ethanol–ether (1:2:2, v/v/v), then with 1 ml chloroform–ethanol–ether–HCl (1:2:2:0.01) solvent. Phospholipids were counted by liquid scintillation as in [7,18]. Phospholipid radioactivity was the sum of the radioactivities extracted by the 2 solvents.

3. Results

Figure 1A, shows the influence of various extracellular Na^+ concentrations on amylase secretion in rat parotid glands non-stimulated or stimulated by 5 μM carbachol, in the presence of 2.5 mM extracellular calcium. In control, reduction of Na^+ to 10 mM results in a sharp increase of the amylase secretion. Similar results were obtained in [8]. In contrast, the amylase secretion enhanced by carbachol is significantly higher than control only at >20 mM Na^+. The net secretion of amylase (difference in amount of amylase released in the presence or absence of carbachol) due to the cholinergic agonist increases linearly until 45 mM Na^+ (see the inset).

Figure 1B shows the effect of sodium on amylase secretion in absence of extracellular calcium. A decrease of Na^+ concentration also evokes an increase in amylase discharge. However, the maximum of amylase output observed at 10 mM Na^+ with calcium is suppressed and the amount of secreted amylase is lowered. The cholinergic agonist still evokes a small stimulation of amylase secretion. But the net secretion due to carbachol increases only at 0.05–5 mM Na^+.

Figure 2 describes the effect of various extracellular Na^+ concentrations on *myo*-[2-^{3}H]inositol amount in TCA-soluble fraction of gland fragments. In the presence or absence of extracellular calcium the *myo*-inositol uptake is increased for Na^+ at >45 mM. The amount of *myo*-inositol in TCA-soluble fraction is decreased by 5 μM carbachol when calcium is present or absent in the incubation medium. A

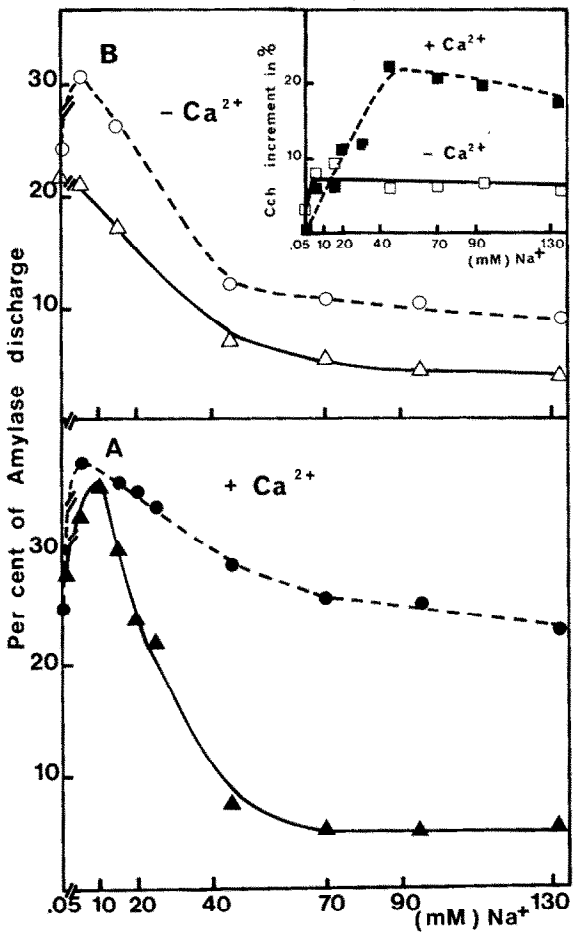

Fig.1. Influence of extracellular Na^+ concentration on amylase discharge induced by carbachol. Glands were incubated at 37°C in Tes–Ringer buffer for 40 min with Na^+ at 0.05–132 mM (see section 2). (A) In the presence of 2.5 mM Ca^{2+}: (▲—▲) control; (●- - -●) 5 μM carbachol. (B) In the presence of 0.1 μM Ca^{2+}: (△—△) control; (○- - -○) 5 μM carbachol. Inset: increment of amylase secretion due to carbachol in the presence (■- - -■) or absence (□—□) of Ca^{2+} as a function of Na^+ concentration. The results were expressed as the mean of 4 separate experiments.

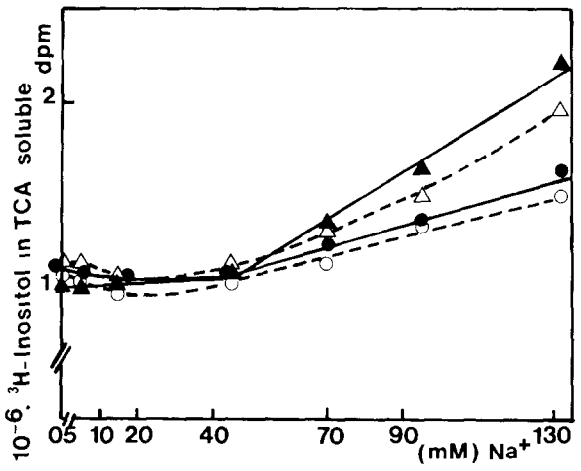

Fig 2 Influence of extracellular Na^+ concentration on *myo*-[2-^{3}H]inositol amount in TCA-soluble fraction Rat parotid glands were incubated in Tes- Ringer medium for 40 min with Na^+ at 0 05–132 mM (see section 2) Results were expressed in dpm g tissue^{-1} In the presence of 2 5 mM Ca^{2+} (● - ●) control, (▲— ▲) 5 μM carbachol In the presence of 0 1 μM Ca^{2+} (○- - - -○) control, (△- - - -△) 5 μM carbachol The results were expressed as the mean of 4 separate experiments

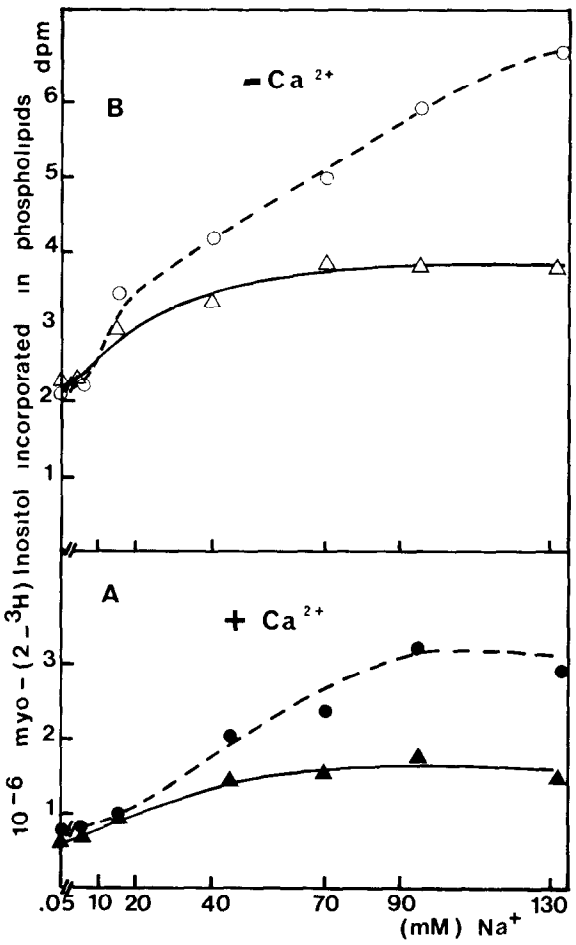

Fig 3 Influence of extracellular Na^+ concentration on the *myo*-[2-^{3}H]inositol incorporation into phospholipids Parotid glands were incubated in Tes–Ringer medium for 40 min with Na^+ at 0 05–132 mM (see section 2) Results were expressed dpm g tissue^{-1} for the same amount of free intracellular *myo*-[2-^{3}H]inositol (TCA-soluble fraction) (A) In presence of 2 5 mM Ca^{2+} (▲— ▲) control, (●- - - -●) 5 μM carbachol (B) In presence of 0 2 μM Ca^{2+} (△—△) control, (○- - - -○) 5 μM carbachol The results were expressed as the mean of 4 separate experiments

similar result was obtained when tissue was treated with 0 1 mM EGTA (unpublished results)

Figure 3 shows the effect of various extracellular Na^+ concentrations on cholinergic stimulation of *myo*-[2-^{3}H]inositol incorporation into phospholipids (mainly into phosphatidylinositol (PI) [7]) Carbachol, 5 μM, stimulates this incorporation The cholinergic effect is both observed in the presence (3A) or absence (3B) of extracellular calcium The incorporation of *myo*-[2-^{3}H]inositol into PI has been shown [7] to increase in the control when the incubation medium is Ca^{2+}-deprived The cholinergic effect on phosphatidylinositol turnover, either in the presence of Ca^{2+}, or absence of Ca^{2+}, is dependent on the extracellular Na^+ concentration In the presence of Ca^{2+} in the incubation medium, the cholinergic effect reaches a maximum level at 90 mM Na^+ However these data are not in agreement with results in [14] who found no effect of Na^+ on the breakdown of phosphatidylinositol by measuring the phosphate content of PI

Figure 4 (curve a) shows the stimulation by 2 5 mM Ca^{2+} of amylase secretion induced by carbachol, as a function of extracellular Na^+ concentration

The amylase discharge due to the extracellular Ca^{2+} and induced by the cholinergic agonist increases linearly until 45 mM Na^+ Figure 4 (curve b) indicates that cholinergic stimulation of phosphatidylinositol turnover is also dependent on the extracellular Na^+ It is interesting to note that cholinergic stimulation of secretory processes (dependent on extracellular Ca^{2+}) and phosphatidylinositol turnover (independent of

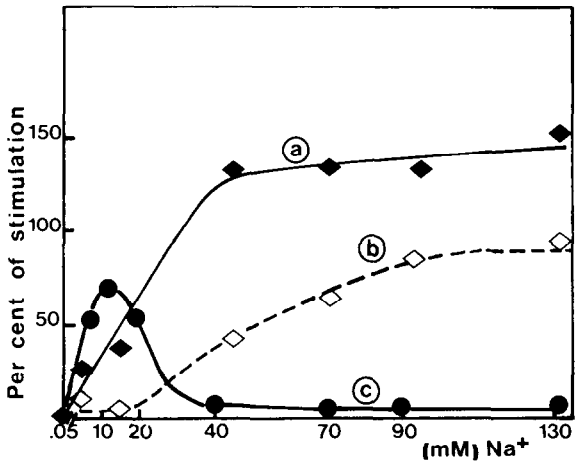

Fig.4. Stimulation of amylase secretion due to extracellular Ca^{2+} in presence (curve a ♦—♦) or in absence (curve c ●- - -●) of carbachol, and of phosphatidylinositol labelling (curve b ◇- - -◇) as a function of Na^+ concentration in extracellular medium. The stimulation of amylase secretion is expressed as:

Curve a

$$\frac{\%\text{ carbachol released amylase (in } Ca^{2+}\text{ medium)}}{\%\text{ carbachol released amylase (in } Ca^{2+}\text{-free medium)}} - 1$$

Curve c

$$\frac{\%\text{ control released amylase (in } Ca^{2+}\text{ medium)}}{\%\text{ control released amylase (in } Ca^{2+}\text{ free medium)}} - 1$$

exogenous Ca^{2+}) are both dependent on the variations of Na^+ concentration gradient. In contrast the curve c shows the stimulation by 2.5 mM Ca^{2+} of amylase secretion in the control.

When Na^+ concentration is lowered to 10 mM in incubation medium, where no phospholipid effect is clearly seen, a high amylase output dependent on extracellular Ca^{2+} occurs, in the absence of cholinergic agonist.

4. Discussion

In rat parotid glands the amylase discharge stimulated by muscarinic cholinergic agonist is dependent on the Ca^{2+} concentration gradient between the extracellular and intracellular medium [1]. This study shows that this process is also dependent on the Na^+ concentration gradient between extra- and intracellular medium. As shown [1], when exogenous Ca^{2+} concentration is lowered to 0.1 μM, the cholinergic agonist still evokes a small amylase output. But this effect seems to be independent of the extracellular Na^+ at >5 mM. However when the incubation is performed at low Na^+ (0.05 mM) the cholinergic stimulation of amylase discharge is almost suppressed in the presence or absence of calcium. These observations are consistent with data in [15] on the exocrine pancreas. Other authors have suggested that the presence of Na^+ is not obligatory in protein release induced by cholinergic agonist, but that the lack of response in Na^+-free medium may be only a consequence of an inhibition of fluid secretion [16]. In our experimental conditions, 10 μM isoproterenol induces the same rate of amylase secretion (59% of amylase content) (results not shown) through the β-adrenergic receptor [13], in the presence or absence of extracellular Ca^{2+}, independently of the presence of extracellular Na^+. This Na^+ control rules out the importance of fluid secretion in amylase release in our experiments.

We may conclude that Na^+ plays a key role in the cholinergic stimulation of amylase secretion due to the extracellular Ca^{2+}. In the absence of cholinergic agonist, the low Na^+ concentration in the incubation medium could lead to the increase of the intracellular Ca^{2+} by an Na^+–Ca^{2+} exchange mechanism, and then lead to the stimulation of the amylase secretion. Our results are in agreement with data in [11,15] demonstrating an increasing $^{45}Ca^{2+}$ influx in a Na^+-free medium.

As reported for other tissues (kidney [17]), it appears that in rat parotid glands the intracellular *myo*-inositol uptake is dependent on a Na^+ concentration gradient between extra- and intracellular medium. When the Na^+ concentration in extracellular medium is >40 mM, the cholinergic agonist decreases the *myo*-inositol uptake in the presence or absence of Ca^{2+} in the extracellular medium. This carbachol effect is similar to that observed when tissue is incubated with 1 mM ouabain (results not shown). This action of oubain is consistent with data on the kidney [17]. From these data it may be postulated that the cholinergic agonist enhances the intracellular Na^+ level. By decreasing the Na^+ concentration gradient, carbachol lowers the inositol uptake (as does ouabain).

Similar results were obtained with the uptake of α-aminoisobutyric acid which is also cotransported with Na^+ in rat parotid glands (unpublished results)

As shown in fig 3,4, the cholinergic stimulation of phosphatidylinositol turnover is dependent on the Na^+ concentration gradient, and a parallelism for the extracellular Na^+ requirement between amylase secretion and phospholipid turnover stimulated by carbachol is observed

From all these data, we suggest that the cholinergic agonist acts on the muscarinic receptor, first by increasing membrane Na^+ permeability independently of Ca^{2+} (effect on inositol uptake in the absence of Ca^{2+}), which could be coupled with phosphatidylinositol turnover As we postulated, an increase of the turnover of phosphatidylinositol could affect the fluidity of plasmalemma membrane and then enhance the entry of Ca^{2+} which triggers the secretory processes requiring Ca^{2+} [7,18,19]

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