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Effects of Salicylate on Insulin and Glucagon Secretion by the Isolated and Perfused Rat Pancreas

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The effects of sodium salicylate, a prostaglandin synthesis inhibitor, on glucose-induced secretion of insulin and glucagon by the isolated perfused rat pancreas have been studied.

Sodium salicylate inhibited both basal (2.8 mM glucose) and stimulated (16.7 mM glucose) insulin release in a dose dependent manner (1, 5 and 10 mM). This inhibition is not interpretable in terms of a simple inhibition of cyclooxygenase by sodium salicylate.

Basal glucagon release was not changed by 1 mM sodium salicylate but the latter partially blocked its inhibition by 16.7 mM glucose. Higher doses of sodium salicylate (5 and 10 mM) inhibited basal glucagon secretion without affecting its response to 16.7 mM glucose. These findings suggest a predominant stimulatory action of endogenous prostaglandins on glucagon release.

Key words: Insulin, Glucagon, Salicylate, Perfused pancreas.

The role of prostaglandins in the regulation of insulin secretion has been examined extensively (24). Although the results from *in vivo* and *in vitro* experiments have led to conflicting views on whether the effects of prostaglandins are stimulatory (10, 11, 12, 15, 19) or inhibitory (2, 22, 23, 26) most studies have suggested that prostaglandins are involved in the regulation of insulin release.

Compared with the amount of information available regarding the actions of prostaglandins on the beta cell, knowledge about their effects on the alpha cell is limited. However, there seems to be agreement from studies in a variety of investigative models that prostaglandins stimulate glucagon secretion (6, 13, 19, 27).

Sodium salicylate and acethyl salicylic acid, inhibitors of endogenous prostaglandins synthesis, have been shown to stimulate insulin secretion, either *in vivo* (16, 18, 21) or *in vitro* (16, 20) experiments. However, VIK-MO *et*

at. (29) have found that sodium salicylate reduced the plasma concentration of insulin. On the other hand, there is scarce information concerning the effects of inhibitors of prostaglandins synthesis on glucagon secretion. Therefore we have studied the effect of sodium salicylate on glucose induced insulin and glucagon release by the perfused rat pancreas.

Materials and Methods

Chemicals. Crystalline porcine and rat insulin and porcine glucagon were from Novo Industri S.A. ^{125}INa from the Radiochemical Center; purified, bovine serum albumin from Behringwerke A.G.; Sephadex G-25 and G-75 from Pharmacia Fine Chemicals, cellulose CF-11 from Whatman Ltd (U.K.); activated charcoal from Sigma Chemical. All other reagents used were of analytical grade from E. Merck.

Male Wistar-Albino rats (250-300 g b.w.) were anesthetized by an intraperitoneal injection of sodium pentobarbital (5 mg/100 g b.w.) and their pancreases isolated and perfused according to the method of GRODSKY *et al.* (5), slightly modified (3). The perfusion medium was Krebs-Ringer-bicarbonate buffer, suplemented with 1 % bovine serum albumin and equilibrated with 95 % O_2 + 5 % CO_2 (pH = 7.4).

The same perfusion pattern was followed in all experiments: After 30 min preperfusion at 2.8 mM glucose (—30 to 0 min), the medium was switched to 16.7 mM glucose (0 to 40 min). Finally, the medium was changed again to 2.8 mM glucose (40 to 50 min). In test-experiments, sodium salicylate was present between minutes —30 to 40.

Perfusion flow rate was kept constant at 2.5 ml/min. Aliquots of the perfusion effluent were collected at 2 min intervals and stored at —20° C until assayed. Insulin and glucagon were radioimmunologically measured with specific antibiodes (30K for pancreatic glucagon).

RIA tracers were prepared from crystalline pork insulin and glucagon by the Chloramine T procedure (9), and subsequently purified by gel-filtration (Sephadex G-25 and G-75) and adsorption (cellulose CF-11) chromatography, respectively. Bound and free hormones were separated with dextran-coated charcoal (8).

Statistical analysis of results was performed by the unpaired Student's t test.

Results

Figure 1 shows that increasing the perfusate glucose concentration from 2.8 to 16.7 mM caused a rapid rise of insulin secretion to a maximum rate which lasted for the duration of the stimulus. Sodium salicylate decreased this insulin response to glucose in a concentration-dependent manner Whereas it dit not modify either basal (2.8 mM glucose) or glucose (16.7 mM) stimulated release at 1 mM, both of them were significantly decreased at 5 and 10 mM ($p < 0.001$). The latter concentration induced a significantly greater decrease of the insulin response to 16.7 mM glucose than that caused by 5 mM ($p < 0.001$), although both doses of sodium salicylate (5 and 10 mM) produced similar effects on basal release.

Glucagon secretory rate was significantly decreased by almost 49.65 % ± 5.82 when the perfusate glucose concentration was changed from 2.8 to 16.7 mM (fig. 2). 1 mM Sodium salicylate did not modify the basal rate of glucagon secretion recorded at 2.8 mM glucose, but it partially prevented its subsequent inhibition by 16.7 mM glucose that produced only a 20.90 % ± 2.53 decrease.

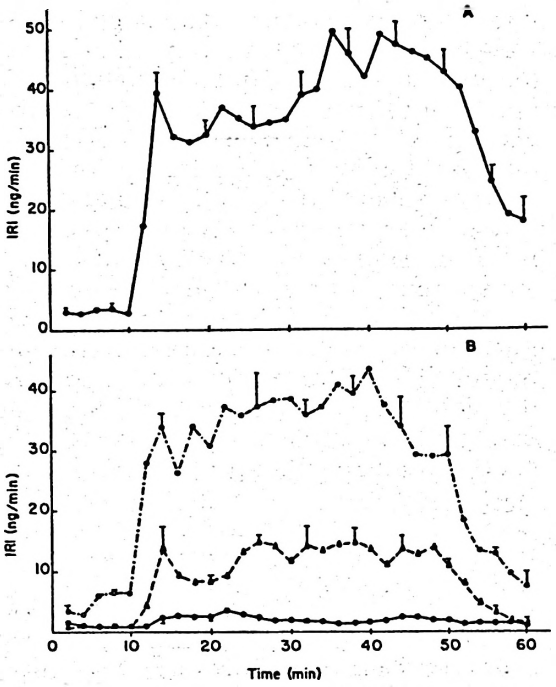

Fig. 1. *Insulin secretory pattern induced by a change of the perfusate glucose concentration from 2.8 mM to 16.7 mM, in the absence (A) or presence (B) of 1 mM (O), 5 mM (Δ) or 10 mM (●) sodium salicylate.*
Means ± S.E.M. of the secretory rates recorded in 6 different experiments are only drawn at 2 min intervals.

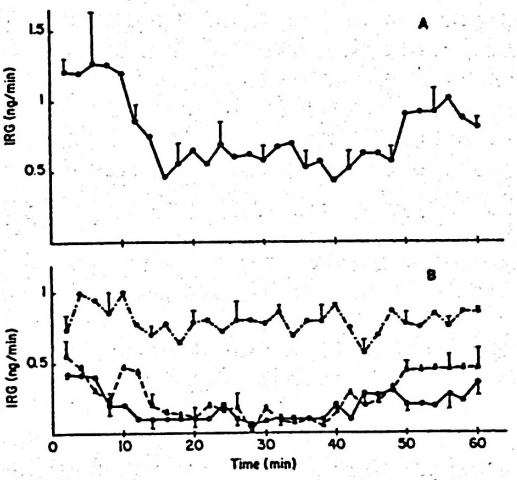

Fig. 2. *Glucagon secretory pattern induced by a change of the perfusate glucose concentration from 2.8 mM to 16.7 mM, in the absence (A) or presence (B) of 1 mM (O), 5 mM (Δ) or 10 mM (●) sodium salicylate.*
Means ± S.E.M. of the secretory rates recorded in 6 different experiments are only drawn at 2 min intervals.

Higher concentrations (5 and 10 mM) of sodium salicylate decreased significantly the basal release of glucagon ($p < 0.001$) which was further diminished by 16.7 mM glucose (47.72 % ± 14.06 and 44.09 % ± 11.76 reduction at 5 and 10 mM sodium salicylate, respectively).

Discussion

Several studies have previously shown that sodium salicylate modifies pancreatic endocrine secretion (4, 16, 29). Some authors attribute its secretory effects to an inhibition of endogenous prostaglandin synthesis (24).

In this work, we demonstrate that sodium salicylate modifies insulin release by the perfused rat pancreas in a concentration dependent manner. Whereas 1 mM sodium salicylate does not affect significantly insulin secretion, it does inhibit either basal or glucose (16.7 mM) stimulated release at higher concentrations (5 and 10 mM). Inhibition of the secretory response to glucose was significantly greater at 10 than 5 mM sodium salicylate. These results are at variance with those reported by GARCÍA *et al.* (4) in the perfused rat pancreas. They found a stimulation of insulin release by 1.87 mM sodium salicylate at 5.5, 11.0 and 16.5 mM glucose and no effect at 2.7 mM glucose. Similarly, METZ *et al.* (16) have reported an stimulatory effect of 1.25 mM sodium salicylate on insulin release which is

dependent on the medium glucose concentration. By contrast, VIK-MO *et al.* (29) have demostrated that sodium salicylate decreases the plasmatic levels of insulin. There seems to be general agreement in relation to the stimulating effects of other inhibitors of prostaglandin synthesis, like acetyl-salicylic acid and ibuprofen, on glucose-induced insulin release, either *in vivo* or *in vitro* experiments (1, 17, 18, 20, 21). However, the use of indomethacin has led to conflicting results (25, 28, 30).

As recently emphasized by MCADAMS *et al.* (14), prostaglandin synthesis inhibitors may exert a dual effect, lower dosis being stimulatory and higher ones inhibitory of glucose-induced insulin release. Within this context, the described effects of sodium salicylate on glucose stimulated secretion by the perfused pancreas migth be attributed to causes other than a general inhibition of prostaglandin synthesis. Firstly, depending on the dose of sodium salicylate, the resulting inhibition of cyclooxygenase may produce variable tissue levels of the different prostaglandins and related derivatives (14). It is known, for example, that prostacyclin (PGI_2) stimulates insulin secretion (7). Secondly, inhibition of cyclooxygenase by sodium salicylate might indirectly increase the lipoxygenase pathway. The 15-hydroxy- and 15-hydroperoxy-5,8,11,13 -eicosatetraenoic acids and 12-hydroxy-5,8,10,14-eicosatetraenoic acid are able to inhibit glucose-induced secretion whereas 5-hydroxy-6,8,11,14-eicosatetraenoic acid stimulates insulin release at a low glucose concentration (31).

The interpretation of the observed effects of sodium salicylate on glucose induced glucagon release is also obscured by the complex interplay between different arachidonic acid metabolites, as commented above for insulin release. However, in this case, all the published experimental data support the view that prostaglandin synthesis inhibitors do generally induce inhibition of glucagon release (13, 14, 19, 27), as it is also shown by our own results. In addition, our finding that increasing doses of sodium salicylate do not modify glucose-induced inhibition of glucagon secretion is supported by very similar results obtained by MCADAMS *et al.* (14) in the perfused rat pancreas using flurbiprofen as a cyclooxygenase inhibitor. Therefore, we may conclude, as the above mentioned authors do, that endogenous prostaglandins predominantly stimulate glucagon secretion.

Resumen

Se estudia el efecto del salicilato sódico, inhibidor de la síntesis de prostaglandinas, sobre las secreciones de insulina y glucagón inducidas por glucosa en el páncreas aislado y perfundido de rata.

El salicilato sódico inhibe tanto la secreción basal de insulina como la estimulada por 16,7 mM glucosa en forma dosis-dependiente (1, 5 y 10 mM). Esta inhibición no es explicable mediante una simple inhibición de la ciclooxigenasa por el salicilato.

La secreción basal de glucagón no es modificada por salicilato sódico 1 mM, si bien éste bloquea parcialmente su inhibición por glucosa 16,7 mM. Dosis mayores de salicilato sódico (5 y 10 mM) inhiben la secreción basal de glucagón sin alterar su respuesta a glucosa 16,7 mM. Estos resultados sugieren una acción predominantemente estimuladora de las prostaglandinas endógenas en la secreción de glucagón.

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