Effects of Salinomycin and Acetylcholine on Salivary Secretion in the Vascularly Perfused Submaxillary Glands of Rats

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Effects of Salinomycin and Acetylcholine on Salivary Secretion in the Vascularly Perfused Submaxillary Glands of Rats

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Summary

Stimulation with salinomycin (0.1 mM) for ten minutes caused a small salivation (peak value: 9.4 ± 2.6 μl/g tissue weight per 10 min), while acetylcholine (5.5 μM) caused a marked salivation (peak value: 117.8 ± 5.8 μl/g tissue weight per 10 min) in the isolated and vascularly perfused submaxillary glands of rats. The salivation evoked by salinomycin and acetylcholine was markedly inhibited by the removal of calcium from the superfusing medium. These results suggest that salinomycin and acetylcholine caused salivation by increasing the intracellular Ca ion concentration in the acinar cells of submaxillary glands of rats.

In the previous report, Katoh and Tsuda (1) showed that carboxylic antibiotics such as salinomycin and monensin caused a dose-dependent amylase increase, which was markedly reduced by the removal of calcium from the superfusing medium, in the parotid segments of sheep and rats.

The present experiment was carried out in order to investigate the effect of salinomycin on saliva secretion using the isolated submaxillary glands of rats which were vascularly perfused with an artificial superfusing solution.

Materials and Methods

Male Wistar-strain rats (230-310 g of body weight) were used. Submaxillary glands were isolated and vascularly perfused according to the reported method (2). Briefly, the rat was anaesthetized by an intraperitoneal injection of urethane (1 g/kg body weight), the ventral midline of the throat was dissected, and the external maxillary artery, the glandular vein and the submaxillary duct were identified. The following procedure of cannulation was carried out under visual control using a binocular magnifier ($\times 2.3$, Vixen). After cannulation of the submaxillary duct with fine polyethylene tubing, the digastric muscle was cut between ligatures, and the fine arterial branches were ligated and cut between ligatures. Then the

external maxillary artery was cannulated with a polyethylene cannula (1 mm O. D., Hibiki) pulled at the tip over a flame to produce a smaller bore ; the oxygenated Tris-buffered superfusing solution (37°C) was perfused through this cannula by using a peristaltic tube pump at a flow rate of 1 ml/min. The glandular vein was also cannulated with a polyethylene cannula after ligation and cutting of fine branches. The gland was removed and transferred into a perfusion bath kept at 37°C. The secretion of saliva was calculated by measuring the changes in fluid length in polyethylene tubing inserted into a duct every ten minutes. At the end of the experiment, the wet weight of the gland used was measured. The composition of Tris-buffered superfusing solution used was similar to that previously described (3). The solution was gassed with pure oxygen. The pH of the solution was 7.4 at 37°C. In a calcium-free solution, calcium chloride was replaced by an equiosmolar amount of sodium chloride containing EGTA (ethylene glycol bis (β-aminoethyl ether)-N, N, N′, N′-tetraacetic acid, 0.1 mM).

Drugs used were salinomycin (Kaken Pharmac., Japan), monensin (Eli Lilly, USA), acetylcholine chloride, EGTA (Wako Pure Chem., Japan) and isoprenaline bitartrate (Sigma, USA). The ionophore was dissolved in ethanol and stored at −20°C. Results are given as the mean±S.E. of the means. For statistical analysis, Student's t-test was employed.

Results

The basal saliva secretion before stimulation in the vascularly perfused submaxillary glands was $0.8\pm0.3\,\mu$l/g tissue weight per 10 min. The time courses of saliva secretion induced by salinomycin and acetylcholine in the control solution or in a calcium-free solution containing EGTA (0.1 mM) are shown in Fig. 1. Stimulation with salinomycin (0.1 mM) or acetylcholine (5.5 μM) for 10 min caused an increase in the saliva secretion. Salinomycin caused a small increase in salivation (peak value: $9.4\pm2.6\,\mu$l/g tissue weight per 10 min), while acetylcholine caused a copious secretion (peak value : $117.8\pm5.8\,\mu$l/g tissue weight per 10 min). The saliva volume evoked by salinomycin was comparable to that evoked by isoprenaline (peak value : $10.9\pm1.3\,\mu$l/g tissue weight per 10 min, not shown), and was larger than that evoked by monensin (peak value : $1.7\pm0.4\,\mu$l/g tissue weight per 10 min, not shown). In a calcium-free solution containing EGTA (0.1 mM), the saliva secretion evoked by salinomycin or acetylcholine was significantly ($P<0.01$) abolished.

Discussion

It is generally accepted that the saliva secretion in the submaxillary and parotid glands of rat is controlled by the cholinergic and adrenergic nerve fibres. The fluid secretion from acinar cells is provoked by the two distinct pathways, e. i., calcium-mediated or cyclic-adenosine-monophosphate-mediated processes (4, 5).

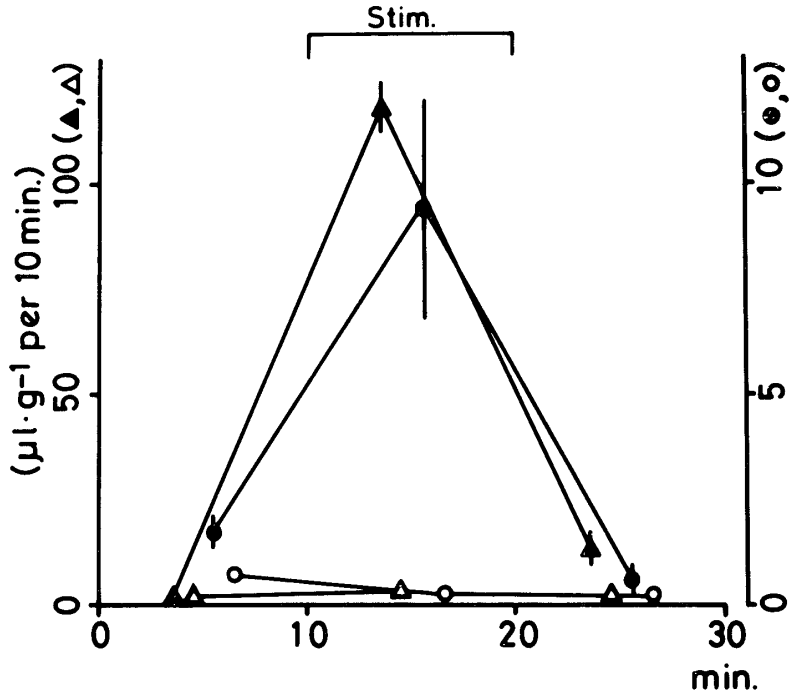

FIG 1. Effects of salinomycin (0.1 mM, ● and ○) or acetylcholine (5.5 μM, ▲ and △) on the saliva secretion in the control solution (closed symbols) or in the calcium-free solution containing EGTA (0.1 mM) (open symbols) in the vascularly perfused submaxillary glands of rats. The stimulation was carried out during the period indicated by the line. Results are given as the mean ± S.E.M. of three to six different experiments.

Acetylcholine, a cholinergic transmitter, exerts its action through the calcium-mediated process. Furthermore, the action of ionophoretic antibiotics such as salinomycin and monensin seems to share a similar process, because the amylase release from the isolated and superfused segments of sheep and rats was reduced by the removal of calcium from the superfusing medium (1).

The results shown in the present experiment provided further evidence supporting the possibility that salinomycin might exert its action on the salivary gland acinar cells by increasing an intracellular calcium ion concentration. Although the action of salinomycin as an ionophore is reported to be for potassium ion (6), our previous (1) and present results showed that the ionophore would be able to transport not only potassium ion but also calcium ion, which is important for biological action.

The reason is not clear why the saliva secretion evoked by salinomycin was smaller than that evoked by acetylcholine. Probably another process in addition to an increase in intracellular calcium concentration might be lacking for saliva formation in the stimulation with the ionophore, or the ionophore might have a side-effect such as inhibition of respiratory process (6).

Acknowledgements

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