SHORT COMMUNICATION

Influence of propranolol on glycoprotein secretion from acinar cells of the rat submandibular gland induced by pilocarpine

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[Accepted for publication: November 13, 1987]

Key words: Rat submandibular gland / secretory segment / saliva / pilocarpine / glycoprotein species

Introduction

Pilocarpine has been widely used as a parasympathomimetic agent, especially in studies of the salivary gland. It had been shown in dogs that the concentrations of total carbohydrate and protein in saliva, elicited from parotid glands by treatment with large doses (2-4 mg/kg) of pilocarpine administered intravenously, are significantly higher than those elicited by low doses (0.4-1.0 mg/kg) of pilocarpine1). Moreover, it has been reported that when large doses of pilocarpine (10 mg/kg) are administered intraperitoneally, levels of both amylase and total protein in saliva elicited from parotid glands of rats are more similar to those in saliva elicited by sympathetic nerve stimulation rather than by parasympathetic nerve stimulation2-5). Furthermore, action of pilocarpine is inhibited by pretreatment with the non-selective β-antagonist, propranolol3-5) or by ectomy of the superior cervical ganglion4). These results suggest that pilocarpine has sympathomimetic action in addition to parasympathomimetic action4).

We reported previously that pilocarpine at a high dose (8 mg/kg, i.p.) stimulated secretion of glycoproteins mainly from acinar cells6). Isoproterenol7), dobutamine7,8), terbutaline9), choline esters9), substance P10) and small doses of dopamine11) have a similar effect.

The present study was carried out to examine the β-adrenergic action of pilocarpine on acinar cells of the rat submandibular glands by measuring flow rate of saliva and the concentration of protein in it, and by comparing electrophoretic profiles of glycoproteins, for saliva evoked in response to pilocarpine at different doses, with or without pretreatment with propranolol.

Materials and Methods

Male Sprague-Dawley rats, eight weeks of age, were deprived of food but given water ad libitum for 24 hr prior to the experiments. The methods for collection of submandibular saliva6,7) and microdissection of secretory segments12) have been described in detail previously. The submandibular saliva was collected at successive intervals of 5 min for 30 min after intraperitoneal administration of pilocarpine at doses of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg. Propranolol, at a dose of 2 mg/kg, was injected intravenously 30 min prior to injection of pilocarpine. The protein content of saliva and secretory segments was determined by the method of Lowry et al.13). One µl of each sample, containing either 0.5 µg or 0.25 µg protein in 1 % (w/v) sodium dodecyl sulphate (SDS), 5% 2-mercaptoethanol, and 20 % glycerol, was applied to a 4-40 % continuous gradient polyacrylamide disc gels in 10 µl capillary tubes, as described by Rüchel.
and subjected to electrophoresis at 60 V for 60 min in 50 mM Tris-glycine buffer (pH 8.4) that contained 0.1% SDS. Each gel was stained with Schiff's reagent (PAS) or with Coomassie Brilliant Blue R-250, and destained in 7% acetic acid. The bands of glycoprotein and protein on the gel were photographed and were also recorded by spectrophotometric traces of the gels made with a Joyce-Loebl 3CS microdensitometer at a wave length of 595 nm, for gels stained with Coomassie Blue, and at 550 nm, for those stained with PAS.

Results and Discussion

The maximal flow rates of submandibular saliva, following intraperitoneal injection of pilocarpine, were recorded 10 to 15 min after injection of doses of 0.5 and 1.0 mg/kg of pilocarpine, and 5 to 10 min after injection of doses from 2 to 8 mg/kg of the drug. When rats were pretreated with propranolol, the maximum flow rates were unchanged at doses of 0.5, 1.0, and 8.0 mg/kg of pilocarpine, but occurred later, after 10 to 15 min, in the case of doses of 2.0 and 4.0 mg/kg of pilocarpine (Fig. 1A, 1B). Both the volume of saliva and the amount of protein secreted from the submandibular gland during the 30-min period after administration of pilocarpine were increased dose-dependently (Fig. 2A). The volume of saliva and the amounts of protein evoked by injections of 0.5 and 1.0 mg/kg of pilocarpine after pretreatment with propranolol did not change significantly, but these parameters decreased in the case of administration of 2 and 8 mg/kg of pilocarpine after pretreatment with propranolol (Fig. 2B). The concentration of protein in saliva evoked by administration of pilocarpine at a dose of 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg was calculated as 1.18±0.18, 0.75±0.10, 1.19±0.09, 1.62±0.09, and 1.62±0.13 mg/ml, respectively. After pretreatment with propranolol these value were altered to 1.78±0.34, 0.74±0.09, 0.77±0.07, 0.73±0.09 and 1.11±0.09 mg/ml, respectively.

Electrophoretic patterns of the glycoproteins which are present in the saliva that is secreted in response to pilocarpine, contain three main bands, I, III, and IV, as shown in Fig.
Fig. 2 Effects of propranolol on total volume of saliva (A) and total amount of protein (B) secreted from rat submandibular glands in response to pilocarpine in different doses.

A: ○ pilocarpine; ● pilocarpine + propranolol.
B: ○ pilocarpine; ● pilocarpine + propranolol. Propranolol at a dose of 2 mg/kg was injected intravenously 30 min prior to intraperitoneal injection of pilocarpine at doses of 0.5-8.0 mg/kg. Each point represents the mean ± S. E. of results from 6 rats. *, ** and *** indicate a significant difference from the results with pilocarpine at P < 0.05, P < 0.01, and P < 0.001, respectively, by Student's t-test.
Fig. 3 Effects of propranolol on profiles of glycoproteins from saliva secreted from rat submandibular glands in response to pilocarpine at different doses. Propranolol at a dose of 2 mg/kg was injected intravenously 30 min prior to intraperitoneal injection of pilocarpine in doses of (A) 0.5 mg/kg, (B) 2.0 mg/kg, and (C) 8.0 mg/kg. Each sample was applied to the gel such that 0.5 µg of protein was loaded on each gel.

The intensity of the glycoprotein band I in saliva evoked by pilocarpine in large doses observed in our data may be due to stimulation of β-adrenergic receptors on the acinar cells by an indirect effect.

Our results appear to present conclusive evidence that pilocarpine activates cholinergic receptors in small doses, but in large doses it activates both cholinergic and β-adrenergic receptors on the acinar cells of the rat submandibular gland.

Acknowledgement

This study was supported in part by a Grant-in-Aid for Scientific Research (No. 60480404) from the Ministry of Education, Science and Culture of Japan.

References
3) Schneyer, C. A.: Modification of the action


