Chloride Concentration of Rat Parotid Saliva Evoked by Electrical Stimulation of Parasympathetic or Sympathetic Nerves with or Without Antagonists (43050)

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Abstract. **Chloride (Cl) of saliva evoked by electrical stimulation of the parasympathetic nerve to parotid gland was from two to seven times higher than that elicited with sympathetic nerve stimulation; [Cl] remained elevated (125–135 mEq/liter) for 60 min of parasympathetic nerve stimulation, whereas Cl of sympathetically evoked saliva decreased from high levels of 58 to 15 to 20 mEq/liter. The administration of propranolol, the β-adrenergic antagonist, 20 min prior to initiation of sympathetic nerve stimulation resulted in saliva with Cl of 100 mEq/liter; when phentolamine, the α-adrenergic antagonist was administered prior to sympathetic nerve stimulation, [Cl] was 48–35 mEq/liter. Values with the β-agonist, isoproterenol, were about 35 mEq/liter, whereas phenylephrine, an α-adrenergic agonist, evoked saliva with Cl ranging from 113 to 85 mEq/liter. Flow rate was very high with parasympathetic nerve stimulation and low with sympathetic nerve stimulation, but [Cl] with β-blockade was not flow dependent: flow was very low but Cl high. Cl secretion is principally regulated by activation of cholinergic and α-adrenergic receptors.** [P.S.E.B.M. 1990, Vol 194]

Secretion of chloride (Cl) from salivary glands in response to autonomimetic stimulation has been assessed to some extent (1–3), but the secretion of Cl in response to electrical stimulation of the autonomic pathways to the gland has been confined chiefly to examination of transport kinetics in perfused main duct of submandibular gland of rat (4–6). In view of the recent work that shows a dysfunction in the β-adrenergically mediated secretion of Cl from cells of sweat ducts and trachea of persons with cystic fibrosis (7, 8), it became important to examine the role of the autonomic nerves and receptors in regulation of Cl secretion from salivary glands, since these organs are also affected in cystic fibrosis. The parotid gland of rat is ideally suited for such studies since the parasympathetic and sympathetic nerves can be stimulated electrically in the presence and absence of autonomic antagonists. Thus, the role of particular receptors as well as the nerves themselves in Cl regulation can be elucidated.

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Materials and Methods

Adult Long-Evans male rats, 3–4 months old, weighing 300–400 g were the experimental animals used. Eighteen hours before acute experimentation, animals were deprived of food but not water. Animals were anesthetized with pentobarbital sodium and injected intraperitoneally with a dose of 50 mg/kg body wt. Additional dosages of anesthetic were given whenever it was necessary to keep the animals in a state of light anesthesia throughout the experiments. They were tracheotomized with polyethylene tubing to provide a clear airway and were kept warm by electric light. For stimulation of the sympathetic innervation, bipolar platinum electrodes were mounted on a manipulator and the tips of the electrodes placed under and around the trunk. The nerve fibers were raised from the surrounding tissues by tips of microelectrodes attached to the manipulator. Thus, the leakage of the electric current to the surrounding tissues was minimized. The auriculotemporal nerve was also isolated and stimulated. For either nerve, a stimulator (Grass Instruments

Co. model SD5) was used to deliver square wave pulses of 4-msec duration at a frequency of 16 pulses/sec and an intensity of 4 V.

To determine effects of α- and β-adrenergic responses, three kinds of experimental animals were prepared. With one group of animals only direct sympathetic nerve stimulation was employed without any blocking agent. In a second group of rats, an α-adrenergic blocking agent, phentolamine (PE) (kindly supplied by Smith, Kline and French Laboratories), was diluted with distilled water and given intraperitoneally at a dose of 3 mg/kg body wt 20 min before the nerve was stimulated. A β-adrenergic blocking agent, propranolol (kindly supplied by Ayerst Laboratories), was diluted with distilled water and administered intraperitoneally at a dose of 3 mg/kg body wt to the third group of animals. Stimulation of either the sympathetic or parasympathetic nerve was continued for 60 min.

The β- and α-adrenergic agonists, isoproterenol (ISO) and PE, were administered intraperitoneally to separate groups of rats in doses of 25 mg/kg body wt (ISO) and 5 mg/kg body wt (PE).

Saliva was collected for 60 min from the freed and cut end of the parotid duct from all animals. Ten-microliter samples were obtained at 10-min intervals and [Cl] subsequently determined by Cl tritator. Secretory rate was determined by relating volume of saliva collected for a timed interval to gland weight (determined by rapid weighing on a torsion balance) and expressed as μl/min/g of gland weight.

For analysis of data, the concentration of Cl or flow rates were expressed as mean ± SE. The difference between means was compared by the standard Student's t test. The difference was considered significant if probability values were less than 0.05.

Results

The data in Figure 1 show the Cl levels of parotid saliva elicited from adult rats by diverse autonomic stimulation during 60 min of stimulation. [Cl] of saliva evoked by electrical stimulation of the parasympathetic nerve to parotid gland was high initially (about 135 mEq/liter) and remained close to 125 mEq/liter for the 60 min of stimulation. In contrast, electrical stimulation of the sympathetic nerve evoked a saliva with a [Cl] of only 58 mEq/liter initially, and even within 10–15 min levels decreased to 35–25 mEq/liter; for the next 45 min, concentration remained between 15 and 20 mEq/liter. With administration of propranolol, the β-adrenergic antagonist, 20 min prior to initiation of sympathetic nerve stimulation, [Cl] of elicited saliva was generally about 100 mEq/liter throughout the 60 min. When phentolamine, the α-adrenergic antagonist was administered 20 min prior to initiation of stimulation of the sympathetic nerve, [Cl] ranged from 48 to 35 mEq/liter.

Administration of high doses of ISO, the β-adrenergic agonist, evoked a saliva with [Cl] similar to that seen with stimulation of the sympathetic nerve (Fig. 1), PE, on the other hand, evoked saliva with [Cl] ranging from 113 to 85 mEq/liter, concentrations similar to

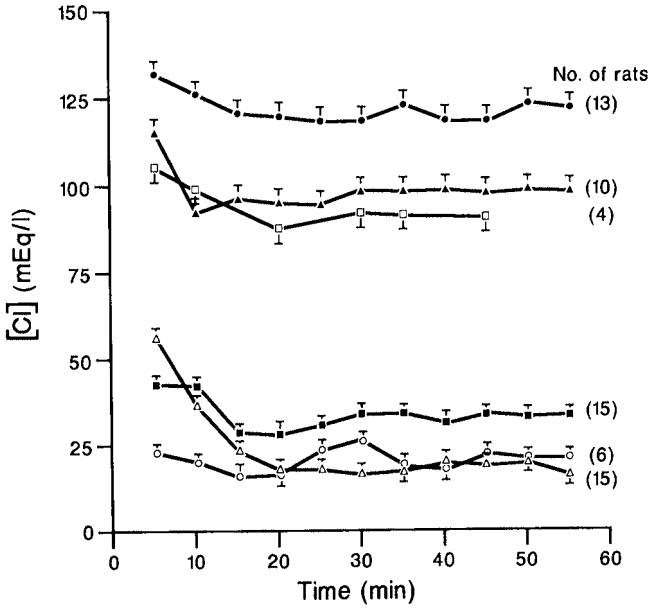

Figure 1. Time course of change in [Cl] of parotid saliva in response to electrical stimulation of either the parasympathetic nerve (●——●), sympathetic nerve (△——△), sympathetic nerve 20 min after intraperitoneal injection of propranolol (3 mg/kg body wt) (▲——▲), or phentolamine (3 mg/kg body wt) (■——■), or in response to intraperitoneal injection of ISO (25 mg/kg body wt) (○——○), or phenylephrine (5 mg/kg body wt) (□——□). Each point is mean ± SE.

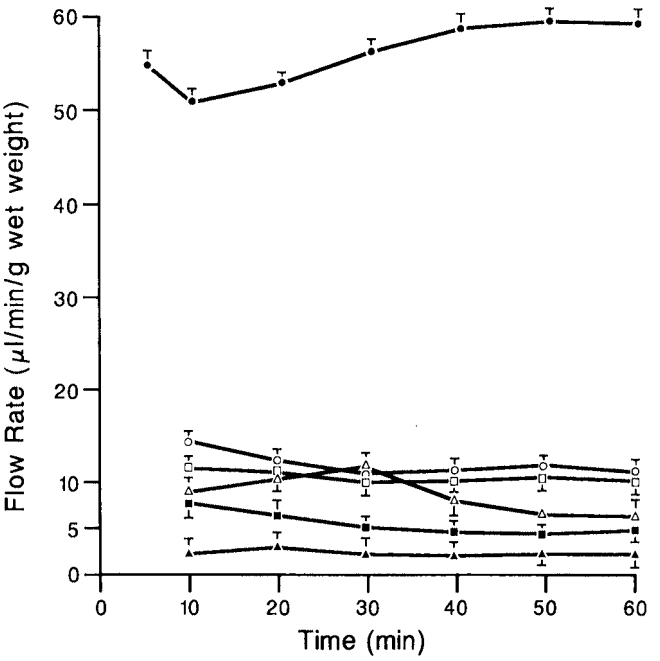

Figure 2. Time course of change in flow rate of parotid saliva under same conditions noted in Figure 1.

those seen with β-blockade and sympathetic nerve stimulation.

Flow rate of saliva elicited by stimulation of the parasympathetic nerve was initially high (54 μl/min/g of gland) and remained high throughout the 60 min (with a low of 50, and high of 60 μl/min/g) (Fig. 2). In contrast, flow rate of saliva evoked by stimulation of the sympathetic nerve was initially 9 μl/min/g, rose to 11 μl/min/g, and fell thereafter to reach a low of 6 μl/min/g; that elicited by ISO (dosage, 25 mg/kg body wt) or phenylephrine (5 mg/kg body wt) was even higher (10–13 μl/min/g). With β-adrenergic blockade by propranolol, the flow elicited with stimulation of the sympathetic nerve was much lower than that observed with sympathetic nerve stimulation alone. That elicited with α-adrenergic blockade by phentolamine was less than that with sympathetic nerve stimulation alone.

Discussion

The [Cl] of parotid saliva evoked by electrical stimulation of the parasympathetic nerve was initially only twice as high as that elicited by electrical stimulation of the sympathetic nerve. Since, however, [Cl] with parasympathetic stimulation remained high throughout the 60 min but rapidly dropped with sympathetic stimulation, it was usually about seven times higher with parasympathetic than with sympathetic stimulation. Blockade of β-adrenoceptors by propranolol during stimulation of the sympathetic nerve elicited saliva with [Cl] which was also high (nearly 100 mEq/liter), but less than that observed with parasympathetic nerve stimulation; levels with β-blockade resembled those evoked by the α-agonist phenylephrine. Thus, when α-adrenoceptors were activated (either with sympathetic nerve stimulation plus β-blockade, or by the α-agonist), [Cl], although high, was nonetheless only 75–85% of auriculotemporal values.

However, the role of α-adrenoceptors was more prominent than that of β-adrenoceptors in regulation of Cl secretion. Activation of β-adrenoceptors resulted in Cl levels that were about 30% of those found in saliva secreted in response to activation of α-adrenoceptors. In submandibular gland, α- and β-adrenoceptors also regulate Cl secretion in whole gland (2) and transport of Cl in perfused main duct (2), but the predominant regulatory effect on Cl transport, with stimulation of the sympathetic nerve is in this instance also the result of activation of α-adrenoceptors (6).

Flow rate of sympathetically elicited parotid saliva was previously shown (9, 12) to be regulated chiefly by activation of β-adrenoceptors. ISO also caused a more copious salivary flow than did phenylephrine, and thus showed that exogenously administered agonists induced responses similar qualitatively to those seen with nerve stimulation and selective adrenergic blockade. These findings are similar to our own (9–11) and Thulin's earlier data (12), and support the view that it is β-adrenoceptor activation of parotid that has the principal regulatory role on flow rate with adrenergic stimulation. Because the dosages of ISO and PE must be high to evoke secretion (13) and because, at such dosages, ISO and PE have affinities for both α- and β-receptors in salivary glands (13), the data on nerve stimulation with selective antagonists provide more reliable evidence for the respective roles of the two adrenoceptors.

The marked difference in flow rate observed with parasympathetic and sympathetic nerve stimulation can account for the marked differences in Cl secretion observed under these two conditions. Moreover, with parasympathetic stimulation, [Cl] is flow dependent, just as [Na] of parasympathetically evoked saliva is flow dependent (14). However, [Cl] with adrenergic stimulation is not strictly flow dependent: Flow rate when α-adrenoceptors were activated (either by phenylephrine or with β-blockade during sympathetic nerve stimulation) was uniformly low, yet [Cl] was quite high. Activation of β-adrenoceptors (with ISO or blockade of α-receptors during sympathetic nerve stimulation) produced saliva with low flow rates and low [Cl]. Thus, with sympathetic nerve stimulation, the characteristics of the salivary flow rate and Cl secretion pattern reflect principally activation of β-adrenoceptors, since with activation of both α- and β-adrenoceptors, the ensuing secretion most closely resembles that observed when only β-adrenoceptors are activated. An inhibitory effect of β-adrenoceptors on α-adrenoceptors (10) may therefore by implied since, in the absence of β-activation (phenylephrine or β blockade) Cl levels increase far above levels seen with sympathetic nerve stimulation (and activation of both α- and β-receptors). Cl secretion is thus not facilitated by β-activation but rather diminished; but the activation of the β-adrenoceptors establishes the normal level of sympathetically elicited secretion.

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