ACTION OF TYRAMINE ON THE SALIVARY AMYLASE SECRETION FROM RABBIT PAROTID GLAND IN REFERENCE TO THAT OF NICOTINE

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The salivary glands, which are innervated by both sympathetic and parasympathetic nervous systems, receive their main secretory innervation from the parasympathetic nervous system (1). It was reported that sympathetic nervous system played an important role in secretion of amylase, a main component of parotid saliva, and that the β receptors were the ones involved in this mechanism (2-5). It was also reported that nicotine caused an increase in amylase secretion as well as salivary flow and suggested that the increasing effect of nicotine on amylase secretion was not due to the direct action on the ganglion, but the action of catecholamine released from adrenal medulla. The acceleration of nicotine in salivary flow, however, was not explained in the same way (6, 7). On the other hand, it was proposed in various organs that sympathetic effect of tyramine was caused by a release of noradrenaline from sympathetic nerve endings (8). It is, therefore, of interest to examine actions of tyramine on amylase secretion in comparison with those of nicotine.

METHODS

Male rabbits weighing 2 to 3.5 kg were anesthetized by the intraperitoneal injection of urethane (1.5 g/kg). Additional small doses of urethane were given when necessary. The trachea was exposed and cannulated, and superior cervical ganglion was removed.

The parotid duct was cannulated using a short piece of stainless steel tubing which was connected to a fine polyethylene tubing with a dead space of approximate 25 μl. The peripheral cut end of auriculotemporal nerve was electrically stimulated using platinum bipolar electrodes with 2 msec pulses at 6 to 10 Hz. The voltage and frequency were adjusted to maintain the salivary flow at 100 to 200 mg per minutes. Each experiment was started after the amylase activity and salivary flow reached a steady state. Each drop of the saliva was counted separately to determine amylase activity and protein concentration (single drop analysis). In some experiments, the postganglionic cervical sympathetic nerve was stimulated with bipolar electrodes. Stimulation was used with electric square wave of 2 msec duration at 15 Hz and voltages sufficient to cause dilatation of the pupil for 30 seconds. Unilateral sympathetic denervation of parotid gland was achieved

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FIG. 1. Schema of innervation of the parotid gland. This preparation has an anatomical characteristic enable to stimulate postganglionic fiber of the parasympathetic nerve.

by removal of a superior cervical ganglion under urethane anesthesia about 2 weeks before the experiment; a sham operation was performed on the opposite side (Fig. 1). Amylase activity was determined by the modification of Bernfeld's method (9, 10) and protein was determined by the method of Lowry et al. (11).

Drugs used were L-nicotine, 1-adrenaline hydrochloride, 1-noradrenaline hydrochloride, tyramine hydrochloride, phenoxybenzamine hydrochloride, propranolol hydrochloride, bretylium tosylate and reserpine. Phenoxybenzamine was injected slowly in geometricaly increasing doses in the range of 1.1, 2.3, 4.6 mg/kg to a total of 8 mg/kg at approximate 10 minute intervals. Bretylium was injected slowly for 3 to 5 minutes. The intervals of injections of tyramine and nicotine were 60 to 90 minutes. All drugs except reserpine were administered intravenously via femoral vein. For reserpinization, 100 µg/kg of reserpine was daily injected subcutaneously for 10 days.

Each result was obtained from 4 to 7 rabbits and each figure illustrated is an example from typical experiments.

RESULTS

Effects of tyramine, nicotine and adrenaline on amylase secretion induced by auriculotemporal nerve stimulation

Intravenous injection of nicotine (300 µg/kg) and adrenaline (3 µg/kg) caused increases in amylase activity and protein concentration of saliva induced by auriculotemporal nerve stimulation and produced an initial acceleration in flow rate as noted in the previous report. Intravenous injection of tyramine (10-300 µg/kg) also caused increases in amylase activity and protein concentration of saliva induced by auriculotemporal nerve stimulation, but did not produce an initial acceleration in flow rate. An acceleration in flow
FIG. 2. Effects of nicotine, adrenaline and tyramine on amylase and protein secretion induced by auriculotemporal nerve stimulation. Effect of nicotine on amylase secretion was revealed later than those of adrenaline and tyramine. Ordinate: Amylase activity ($\times 10^3$ u/g of saliva, $\bullet$: amylase activity of a drop), Protein concentration (mg/g of saliva, $\circ$: protein concentration of a drop) and flow rate (mg/min, dotted line). Abscissa: Time scale was taken arbitrarily so as to be equal interval of drops on the figure.

FIG. 3. Effect of phenoxybenzamine on the increasing effect of tyramine on amylase and protein secretion induced by auriculotemporal nerve stimulation. Phenoxybenzamine did not inhibit the increasing effect of tyramine on amylase and protein secretion. See Fig. 2. for explanation of superscripts.
rate started approximate 15 seconds after intravenous injection of adrenaline, and increases in amylase activity and protein concentration did about 20 seconds and reached a maximum 50 to 60 seconds. Approximate 15 seconds after the administration of nicotine, an acceleration in flow rate began. Increases in amylase activity and protein concentration began 35 to 40 seconds after the administration of nicotine and reached a maximum at 70 to 80 seconds. In a case of tyramine, the increase in amylase and protein secretion started about 15 seconds after the administration and reached a maximum 55 to 60 seconds. Increases in amylase and protein secretion with nicotine responded 15 to 20 seconds later than those with adrenaline and tyramine (Fig. 2).

Influences of blocking agents on tyramine and nicotine effects on the amylase secretion induced by auriculotemporal nerve stimulation

Phenoxybenzamine: After the treatment with phenoxybenzamine, amylase activity and protein concentration of saliva in the steady state were increased 1.5 to 2 fold of control in 7 out of 11 experiments. The increases in amylase and protein secretion produced by nicotine and tyramine were not inhibited by the pretreatment with phenoxybenzamine (8 mg/kg) 30 minutes beforehand (Fig. 3).

Propranolol: The increases in amylase and protein secretion produced by nicotine and tyramine were remarkably inhibited by the pretreatment with propranolol (0.3 mg/kg) (Fig. 4).

Bretylium: Amylase activity and protein concentration were markedly increased by bretylium (10 mg/kg), but flow rate was not altered. The increases in amylase and protein secretion produced by tyramine were not inhibited by the pretreatment with bretylium (Fig. 5). The increasing effects of nicotine and noradrenaline were not inhibited

![Fig. 4. Effect of propranolol on the increasing effect of tyramine on amylase and protein secretion induced by auriculotemporal nerve stimulation.](image)
Fig. 5. Effect of bretylium on tyramine effect on amylase and protein secretion induced by auriculotemporal nerve stimulation. No inhibition was observed after bretylium. Response due to noradrenaline was usually somewhat augmented. The increases in amylase and protein secretion and the acceleration in flow rate produced by the electric stimulation of sympathetic nerve were inhibited by the pretreatment with bretylium.

Fig. 6. Influence of adrenalectomy on tyramine effect on amylase and protein secretion induced by auriculotemporal nerve stimulation. No alteration was observed after the adrenalectomy.
Effects of tyramine and nicotine on the amylase secretion in adrenalectomized, denervated and reserpinized animals

Adrenalectomy: Sixty minutes after removal of adrenal glands, nicotine did not show any increase in amylase and protein secretion. The initial acceleration in flow rate produced by nicotine was not significantly altered. On the other hand, the increases in amylase and protein secretion produced by tyramine did not diminish after the removal of adrenal glands (Fig. 6).

Sympathetic denervation: In chronic sympathetic denervated gland, amylase activity and protein concentration of saliva in the steady state were about 1.5 fold higher than those in contralateral control gland of the same animal. The initial acceleration in flow rate and the increases in amylase and protein secretion produced by nicotine were obviously augmented. The increases in amylase and protein secretion produced by adrenaline were also augmented. On the contrary, the increases in amylase and protein secretion produced by tyramine were remarkably inhibited (Fig. 7).

Reserpinization: In reserpinized rabbits, the increases in amylase and protein secretion produced by nicotine were not remarkably altered as compared with those in normal rabbits, while those produced by tyramine were statistically observed to be diminished. Dose-response curves of tyramine and nicotine in reserpinized animals were obtained (Figs. 8 and 9).
FIG. 8. Effect of nicotine on amylase and protein secretion induced by auriculotemporal nerve stimulation after the reserpinization. Rate of increase is expressed as a value obtained by dividing an average of three maximum amylase activities and protein concentrations after nicotine by those before nicotine. Vertical lines indicate the S.D. of the mean. Significant alteration of nicotine effect was not observed after the reserpinization.

FIG. 9. Effect of tyramine on amylase and protein secretion induced by auriculotemporal nerve stimulation after the reserpinization. Rate of increase is expressed as a value obtained by dividing an average of three maximum amylase activities and protein concentrations after tyramine by those before tyramine. Vertical lines indicate the S.D. of the mean. The increasing effect of tyramine was significantly diminished after the reserpinization.

DISCUSSION

Effects of tyramine, nicotine and other sympathomimetic agents on amylase activity and protein concentration of saliva, and salivary flow rate in the steady state during the stimulation of auriculotemporal nerve were observed by means of single drop analysis.
As well as nicotine or adrenaline, tyramine also caused increases in amylase and protein secretion. However, tyramine did not show an initial acceleration in flow rate. The increases in amylase and protein secretion with tyramine responded at the same time as those with adrenaline, while the increases with nicotine did 15 to 20 seconds later than those with adrenaline or tyramine. From this phenomenon, it may be considered that the increasing action in amylase secretion due to tyramine is different from that due to nicotine. Increases in amylase secretion produced by tyramine and nicotine were inhibited by propranolol, but not phenoxybenzamine. In previous reports (3, 5), it was reported that the order of potency in producing an increase in amylase secretion was isoprenaline, adrenaline and noradrenaline, and that propranolol and DCI suppressed the increase in amylase secretion produced by various adrenergic stimuli, while tolazoline and phenoxybenzamine did not, and proposed that sympathetic nervous system was important in the regulation of amylase secretion and that the $\beta$ receptors were the ones involved in this mechanism. It may be considered that as well as nicotine, the effect of tyramine on amylase secretion is mediated through catecholamine in adrenergic receptor level. The stimulating effect of bretylium on amylase secretion, similar to its sympathomimetic effects in various organs, may be sympathomimetic one, which is mainly attributable to a peripheral release of catecholamines from adrenergic tissues (12). After pretreatment with bretylium, the increases in amylase secretion and flow rate produced by sympathetic nerve stimulation were remarkably inhibited, but the increases in amylase secretion produced by noradrenaline, tyramine and nicotine were not. Moreover, the responses to noradrenaline were somewhat augmented. The catecholamine hypersensitization by bretylium was reported in various organs (13). Bretylium inhibits effects of sympathetic nerve stimulation, but not a release of catecholamine from adrenal glands. Boura and Green (13) described that depending upon the test situation, bretylium might increase or decrease the responses to sympathomimetic amines that act by releasing endogenous catecholamines and also described that catecholamine release with tyramine was apparently suppressed by bretylium on the assumption that sensitivities to the catecholamines released by tyramine were increased. According to the assumption, it may not be considered from the present experiment that catecholamine release with tyramine and

<p>| Table 1. Influences of various treatments on the increasing actions of nicotine and tyramine on the amylase secretion by auriculotemporal nerve stimulation from rabbit parotid gland. |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Nicotine</th>
<th>Tyramine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenoxybenzamine</td>
<td>8 mg/kg i.v.</td>
<td>not inhibited</td>
<td>not inhibited</td>
</tr>
<tr>
<td>Propranolol</td>
<td>300 $\mu$g/kg i.v.</td>
<td>blocked</td>
<td>blocked</td>
</tr>
<tr>
<td>Bretylium</td>
<td>10 mg/kg i.v.</td>
<td>not inhibited</td>
<td>not inhibited</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td></td>
<td>blocked</td>
<td>not inhibited</td>
</tr>
<tr>
<td>Sympathetic denervation</td>
<td></td>
<td></td>
<td>inhibited</td>
</tr>
<tr>
<td>Reserpine</td>
<td>100 $\mu$g/kg s.c. for 10 days</td>
<td>unchanged</td>
<td>inhibited</td>
</tr>
</tbody>
</table>

Nicotine: 300 $\mu$g/kg i.v. Tyramine: 100 $\mu$g/kg i.v.
nicotine is apparently suppressed by bretylium. Noradrenaline hypersensitization was not so much observed. Bretylium intervenes in releasing of noradrenaline elicited by nerve action potential from intragranular mobile pool II to extraneural space or cytoplasmic mobile pool I, where noradrenaline is easily displaced by tyramine. The removal of adrenal glands almost completely abolished the stimulating effect of nicotine on amylase secretion, but not that of tyramine. Muscholl (8) described that most authors failed to detect an amine release by tyramine from medullary and extramedullary chromaffin tissues. Robinson (14) indicated that an injection of tyramine into the medium perfusing isolated dog adrenals increased the rate of secretion of catecholamines, and that intravenous pressor dose of tyramine in anesthetized dogs caused a decrease in catecholamine content of adrenal venous blood. Weiner et al. (15) found that, although pressor dose of nicotine increased catecholamine output from the adrenal glands in dogs, equipressor dose of tyramine failed to do so. Results in the present experiment may indicate that a release of catecholamine from adrenal glands is not essential and probably does not play so much significant role in the tyramine-induced amylase secretion. Harakal et al. (16) reported that hemodynamic responses elicited by tyramine were not dependent upon the presence of adrenal cortex or medulla. In chronic sympathetic denervated gland, amylase activity in the steady state was about 1.5 fold higher than that in acutely denervated gland, and the amylase secretion was hypersensitive to catecholamine in accordance with a law of denervation proposed by Cannon (17). The result may show that amylase secretion is predominantly innervated by sympathetic nervous system. The increase in amylase secretion produced by tyramine was remarkably inhibited by chronic sympathetic denervation, by which noradrenaline in sympathetic nerve ending was considered to be depleted. On the other hand, the increase produced by nicotine was somewhat augmented. It seems that tyramine-induced amylase secretion is preceded by a release of catecholamine from sympathetic nerve terminal. In reserpinized rabbits, the increases in amylase and protein secretion produced by tyramine were diminished as compared with those in normal rabbits, while the increases in amylase and protein secretion produced by nicotine were not remarkably altered. The reserpinized rabbits were obtained by injections of 0.1 mg/kg of reserpine daily for 10 days and the general condition of the rabbit was reasonably appropriate. Remarkable decrease in nicotine-induced amylase secretion by reserpinization was not observed. Catecholamine in adrenal medulla in contrast to that in other peripheral tissues is not markedly depleted by reserpinization (18). In previous reports (6, 7), it was reported that no difference in nicotine effects was observed between the acutely denervated gland and the acutely decentralized one. From the results as represented Table 1, the action of nicotine in increasing the amylase secretion was indicated to be neither an action on ganglion nor on nerve terminal of cervical sympathetic nerve, but an indirect action of catecholamine released from adrenal medulla. The increase in amylase secretion with nicotine responded 15 to 20 seconds later than that with adrenaline and tyramine. This latency of the nicotine response in amylase secretion might be due to the circulation time of catecholamines released from adrenal medulla to the parotid gland. It
is, therefore, concluded that tyramine-induced amylase secretion is mediated through catecholamine released from peripheral nerve terminal, while nicotine-induced amylase secretion is mediated through catecholamine from adrenal glands. The results obtained in the experiments concerning denervation, adrenalectomy and reserpinization corroborate the difference of actions between nicotine and tyramine.

SUMMARY

The present experiment was mainly carried out to clarify difference in mechanism of actions between nicotine and tyramine on amylase and protein secretion induced by auriculotemporal nerve stimulation in rabbit parotid glands. The increases in amylase secretion produced by tyramine and nicotine were inhibited by propranolol, but not by phenoxybenzamine. Bretylium did not inhibit the actions of either tyramine or nicotine. Sympathetic denervation predominantly inhibited the action of tyramine, but not that of nicotine. In reserpinized rabbits, the increasing action of nicotine was not suppressed, but that of tyramine was. Adrenalectomy showed a complete inhibition of the nicotine action, but the action of tyramine still remained. From these results, the effect of tyramine on amylase secretion is due to catecholamine released from the store of sympathetic nerve terminal, while that of nicotine is due to catecholamine released from adrenal glands.

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