Effects of Varying Frequency of Sympathetic Stimulation on Chloride and Amylase Levels of Saliva Elicited from Rat Parotid Gland with Electrical Stimulation of both Autonomic Nerves (43197)

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Abstract. Simultaneous stimulation of the parasympathetic and sympathetic nerves to the parotid gland of rats elicited saliva at a rate dependent on the frequency of sympathetic stimulation when parasympathetic frequency was maintained at 16 Hz. The flow rate was lowest at 2 Hz (sympathetic), moderate at 5 Hz, and highest at 16 Hz. Cl concentration of the saliva evoked with stimulation of both nerves was highest at the highest frequency and flow rate (maintained at the level of 102 mEq/liter, for 35 min) and lowest at 2 Hz (declining from 40 mEq/liter initially to 28 mEq/liter). With sympathetic nerve stimulation alone, Cl concentration ranged from 27 to 58 mEq/liter when frequency was varied from 2 to 16 Hz, and with parasympathetic stimulation alone (16 Hz), it ranged from 132 to 124 mEq/liter. Amylase concentration of sympathetically elicited saliva was, in contrast, highest at 2 Hz (1.5 times the level at 5 Hz, and twice the level at 16 Hz), and nearly 18–38 times that seen with parasympathetic stimulation alone. The same pattern was found when both nerves were stimulated, and at 2 Hz (sympathetic), amylase concentration was 1.6 times the level at 5 Hz and 2.6 times the level at 16 Hz. When the two nerves were simultaneously stimulated, the total amount of amylase secreted over 35 min was twice as high as that observed with sympathetic nerve stimulation alone, at any frequency. The relation of frequency to norepinephrine concentration was examined. There was no consistent difference in norepinephrine concentration related to variation in frequency of sympathetic stimulation. Only when both nerves were stimulated at 16 Hz was there a statistically significant reduction in norepinephrine concentration of 46%. A relation between frequency of sympathetic stimulation, flow rate, amylase concentration, and Cl concentration was established, but these changes could not be directly correlated with quantitative differences in norepinephrine concentration. [P.S.E.B.M. 1991, Vol 196]

Activation of both branches of the autonomic innervation to organs normally occurs with physiologic stimulation, but in most organs the effects of the sympathetic and parasympathetic nerves are generally antagonistic (1). The salivary glands are a notable exception to this, and salivary secretion is elicited with stimulation of either branch (2). However, the quantity of saliva and its chemical composition (3) specifically reflect the influence of each branch. Thus, high levels of protein are secreted in response to sympathetic stimulation (3), and low levels with parasympathetic stimulation (3). Electrolyte concentrations also reflect differences in the influence of the two branches (3). However, whereas the effects of separate stimulation of each autonomic pathway have been described (4), data on simultaneous activation of both nerves are sparse (5, 6). Secretion of amylase has been reported with feeding (involving presumably activation of both pathways) (7, 8) and electrical stimulation of both (3,

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5, 6), but the effect of varying levels of activity in one pathway while a constant level of activity is maintained in the other has not been fully examined. It is also known that the kind of stimuli used to activate the nerves determine the level of sympathetic and parasympathetic activity (9). In the present work, the activity of the sympathetic pathway was varied (by using variable frequency of stimulation), but that of the parasympathetic was kept constant. The Cl, amylase, and flow rate were measured in relation to this, and related to glandular levels of the neurotransmitter, norepinephrine (NE).

Materials and Methods

Female Long-Evans rats (4 months of age) used in these experiments were maintained on Purina Chow and water except for 18 hr prior to acute experimentation when food but not water was removed. The rats were anesthetized with sodium pentobarbital (50 mg/kg body wt, ip); the sympathetic and parasympathetic innervations to the parotid gland then were exposed, and bipolar electrodes were placed against each nerve. A Harvard stimulator was used to deliver stimuli that were 4 msec in duration and voltage of 4 V; frequency for parasympathetic stimulation was maintained at 16 Hz for all experiments, whereas frequency of sympathetic stimulation was varied, being either 2 Hz, 5 Hz, or 16 Hz. Flow rate of parotid saliva at each combination of sympathetic and parasympathetic stimulation was calculated by measuring microliters of saliva secreted per minute and relating this to gland weight (determined after each experiment using torsion balance). Saliva was collected directly from the cut parotid duct, and 1- or 10-μl samples were saved for analysis of amylase and Cl. Amylase concentration of the salivary samples (1 μl) was determined according to the method of Myers *et al.* (10); Cl concentration was measured by titration of 10-μl samples of saliva (Haake-Buchler chloridometer). NE was determined on glands obtained in separate experiments. The level of NE was analyzed using a modification of a method described by Krstulovic (11), employing high-performance liquid chromatography and electrochemical detection. The separation was achieved with a Supelco $C_{18}RP_3$ μM column and a Waters pump. The mobile phase, a phosphate-citric acid buffer (0.347 M KH_2PO_4 and 0.03 citric acid), contained 3 mM sodium acetyl sulfate as an ion-pair and methanol (14%) as a modifier. The eluting compounds were detected with a Bioanalytical Systems ampermometric detector set at a potential of +0.5 V. Student's t test was used for statistical analysis of the data.

Results

The data in Figure 1 show flow rate of parotid saliva elicited during 35 min of continuous electrical stimulation of both the parasympathetic and sympathetic nerves; frequency of stimulation to the parasympathetic nerve was maintained at 16 Hz; as the frequency of sympathetic nerve stimulation was varied from 2 to 16 Hz, the flow rate of elicited saliva varied. At 2 Hz, sympathetic, and 16 Hz, parasympathetic, flow rate was initially low (less than 5 μl/min/g) and remained at this level for the 35-min period of stimulation. In the group in which frequency of sympathetic stimulation was 5 Hz, the flow rate was between 8 and 10 μl/min/g throughout the 35-min period of stimulation. When frequency of stimulation of the sympathetic nerve was increased to 16 Hz, flow rate during the first 15 min was 22–25 μl/min/g, but fell to levels of 20 μl/min/g thereafter. Flow rates of sympathetically (16 Hz) and parasympathetically (16 Hz) elicited saliva are also shown in Figure 1. Flow rate of sympathetically elicited saliva was between 4 and 8 μl/min/g during the 35 min of stimulation, and with parasympathetic alone (16 Hz) it was 54–56 μl/min/g throughout the 35 min of stimulation (Fig. 1).

Data in Figure 1 show that the Cl concentration of saliva elicited with simultaneous stimulation of the two nerves was uniformly high when frequency of sympathetic and parasympathetic stimulation was 16 Hz, and varied little from initial concentrations of 102 mEq/liter throughout the 35-min period of stimulation; in rats in which frequency of sympathetic stimulation was 5 Hz (16 Hz parasympathetic), Cl concentration was initially 95 mEq/liter but fell to levels of approximately 80 mEq/liter for the remaining period of stimulation. In rats in which frequency of sympathetic stimulation was only 2 Hz and parasympathetic, 16 Hz, Cl concentration fell from initial levels of 40 mEq/liter, gradually reaching a concentration of 24 mEq/liter at 35 min. With sympathetic nerve stimulation alone, initial Cl concentrations of saliva were as follows: 2 Hz, 27 mEq/liter; 5 Hz, 38 mEq/liter, and at 16 Hz, 58 mEq/liter (not shown in Fig. 1). As shown by data in Figure 1, [Cl] fell from the initial high levels, and between 15 and 35 min [Cl] was 26–22 mEq/liter (all frequencies). With parasympathetic stimulation alone (16 Hz), Cl concentration was initially 132 and fell thereafter to 124 mEq/liter.

Amylase concentration of sympathetically elicited secretion was highest at the lowest (2 Hz) frequency, being 1.5 times the level observed at 5 Hz, and twice the levels observed at 16 Hz (Table I and Fig. 2). In contrast, amylase concentration of parasympathetically elicited saliva (frequency 16 Hz) was about 21 mg/μl throughout the 35 min of stimulation. Thus, at the lowest frequency of sympathetic stimulation, amylase concentration of saliva was 38 times greater than that observed with parasympathetic stimulation. When both nerves were simultaneously stimulated, mean amylase concentration of the saliva was again highest at the

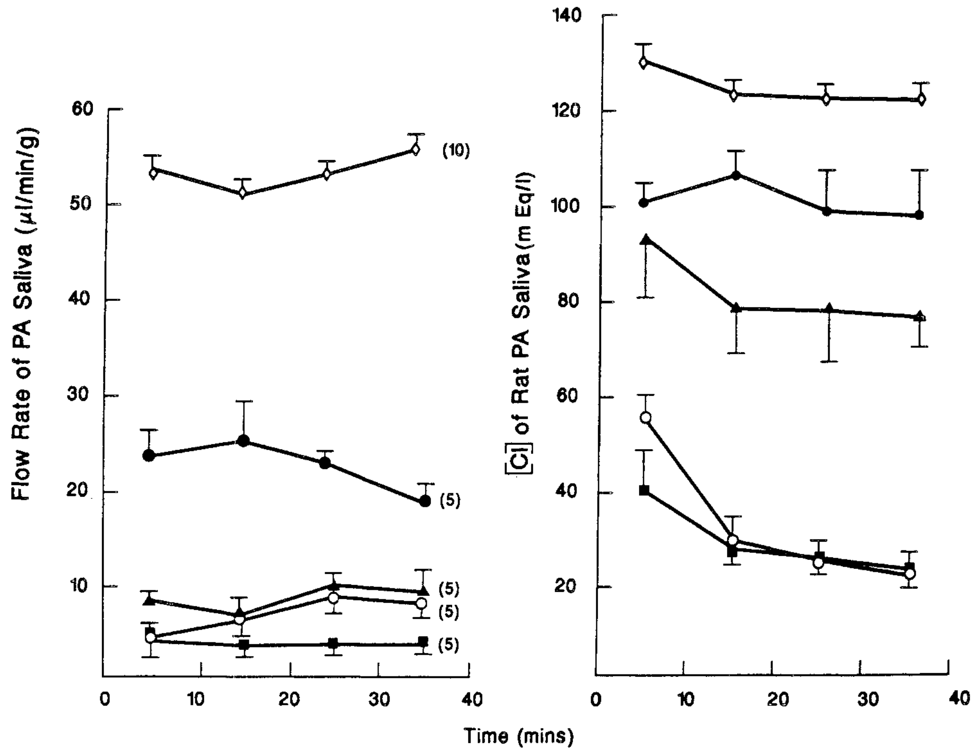

Figure 1. Time course of change in flow rate of parotid saliva in response to electrical stimulation of the sympathetic nerve at frequencies of 2, 5, or 16 Hz in combination with electrical stimulation of the parasympathetic nerve at 16 Hz. Symbols are as follows: sympathetic nerve stimulation at 2 Hz, parasympathetic at 16 Hz, ■——■; sympathetic nerve stimulation at 5 Hz, parasympathetic at 16 Hz, ▲——▲; sympathetic nerve stimulation at 16 Hz, parasympathetic at 16 Hz, ●——●; sympathetic nerve stimulation alone, 16 Hz, ○——○; parasympathetic nerve stimulation alone, 16 Hz, ◇——◇. Numbers in parentheses indicate number of rats and are same for amylase. Time course of change in Cl concentration of parotid saliva uses same symbol designation.

Table I. Effects of Simultaneous or Individual Stimulation of Sympathetic and Parasympathetic Nerves to Parotid Gland on Amylase and Volume of Saliva, with Constant Frequency for Parasympathetic (16 Hz) and Varying Frequency of Sympathetic (2, 5, 16 Hz) Stimulation[a]

Kind of nerve stimulation and frequency[b] (Hz)		Volume (μl/min)	Volume/35 min (μl/35 min)	Amylase concentration (mg/μl)	Total amylase secreted in 35 min (mg/μl × total μl/35 min)
Sympathetic	+ parasympathetic				
2	16	2 ± 0	74	591 ± 45[c]	43,734[c]
5	16	4 ± 0	140	369 ± 70[c]	51,660[c]
16	16	9 ± 2	315	231 ± 38[c]	72,765[c]
2	0	1 ± 0	25	804 ± 31[c]	20,100
5	0	1 ± 0	46	539 ± 39[c]	24,794[c]
16	0	2 ± 0	81	393 ± 28[c]	31,833[c]
0	16	12 ± 2	420	21 ± 2[d]	8,820[d]

[a] Values are mean ± SE. Volumes based on measurements at 10-min intervals over 35 min with total volume calculated from mean flow rates; amylase values are mean values of four- to six 10-min samples from each of five rats.

[b] Parasympathetic nerve stimulated at a constant frequency of 16 Hz, whereas sympathetic nerve stimulation was varied from 2 to 16 Hz; 0 indicates no activity of nerve specified.

[c] Indicates value differs significantly ($P < 0.01$) from each of the two others within a group.

[d] Indicates value differs significantly ($P < 0.01$) from all other values.

lowest frequency of sympathetic stimulation and was 1.6 times the level observed at 5 Hz, and 2.6 times the level at 16 Hz.

The total output of amylase in saliva secreted over 35 min, however, was considerably greater when the two nerves were simultaneously stimulated than when either nerve was stimulated alone. With dual stimulation, total amylase output of the saliva collected over 35 min was, at each frequency of sympathetic nerve stimulation, about twice as high as that observed when the sympathetic nerve was stimulated alone (Table I). Even at the frequency of 16 Hz, however, total amylase

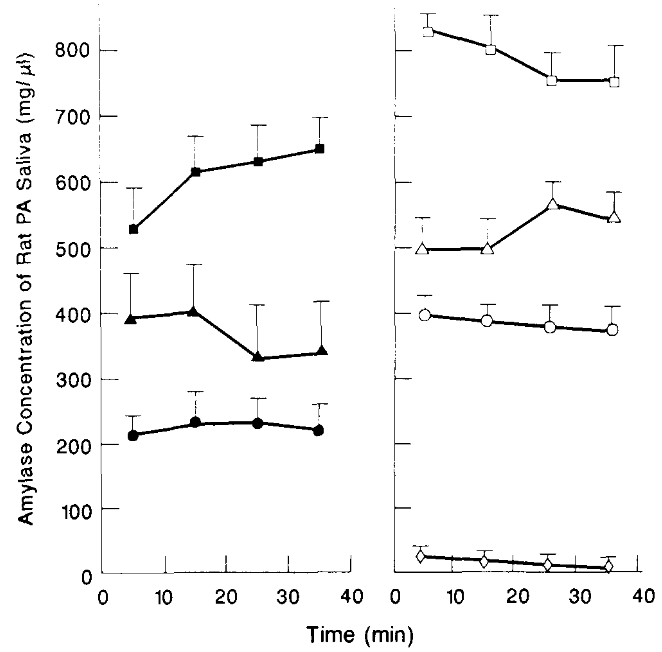

Figure 2. Time course of change in amylase concentration of parotid saliva in response to electrical stimulation of the sympathetic nerve alone at frequencies of 2, 5, or 16 Hz, or in combination with parasympathetic stimulation at 16 Hz. Symbols for dual stimulation as given above; additionally, symbols for sympathetic nerve stimulation alone are as follows: 2 Hz, □——□; 5 Hz, △——△; 16 Hz, ○——○. parasympathetic alone (16 Hz), ◇——◇.

elicited over 35 min of parasympathetic stimulation was considerably less than that observed at any frequency of sympathetic stimulation, either alone or in combination with parasympathetic stimulation. At the lowest sympathetic level (2 Hz, no parasympathetic), total output of amylase in the saliva secreted over 35 min was 2.3 times higher than that of parasympathetic saliva, and at the highest sympathetic level, 16 Hz, 3.6 times greater. With dual stimulation (16 Hz for both nerves), total amylase output reached a level that was 8 times that observed with parasympathetic nerve stimulation at 16 Hz.

NE concentration of parotid glands was measured after 35 min of stimulation of both nerves, or with stimulation of the sympathetic nerve alone. NE levels after 35 min of sympathetic stimulation alone were, at each frequency (2, 5, and 16 Hz), not statistically different from unstimulated control glands (Table II) ($P > 0.01$). Simultaneous stimulation of the sympathetic and parasympathetic nerves did not result in statistically significant ($P > 0.01$) differences at frequencies of 2 and 5 Hz (sympathetic); the only reduction that was statistically significant was the change (46% reduction) ($P < 0.01$) at 16 Hz.

Discussion

Earlier work showed that administration of the β-adrenergic agonist, isoproterenol, even at a dosage too low to elicit secretion, caused a marked elevation in amylase concentration of parasympathetically elicited secretion (12). This showed that even when there was no overt fluid secretion, adrenergic stimulation caused a copious release of amylase that far exceeded that induced by cholinergic stimulation (12, 13). Present data provide quantitative evidence for the role of the adrenergic innervation in regulation of salivary amylase. Thus, the concentration of amylase in saliva elicited when the sympathetic nerve was stimulated alone, or in combination with stimulation of the parasympathetic nerve, varied as a function of frequency of stimulation of the sympathetic nerve.

On the basis of earlier work on sympathetic control of electrolyte transport in perfused main submandibular duct (14), it was assumed that these frequency-related effects were a consequence of differences in amounts of NE available at each frequency of stimulation. These differences in NE resulted in activation of specific adrenoceptors, and at low frequency (and ostensibly, low NE) predominantly β-adrenoceptors were activated, and at high frequency (and high NE), activation of α-adrenoceptors was predominant (14). However, no frequency-dependent reductions in [NE] were established by present data. It is possible that present NE measurements are unreliable indices of the amount of NE released with sympathetic stimulation, since measurements of glandular [NE] are admittedly only an indirect indicator of neurotransmitter release (15). Nonetheless, even though frequency-dependent changes in NE could not be established, changes in flow rate, [Cl], and amylase concentration were related to frequency of sympathetic nerve stimulation.

The predominant effect of varying frequency of sympathetic stimulation appears to be on flow rate, and [Cl] and amylase concentration then vary as a function of flow rate. With high rates of flow, the time for reabsorption of Cl in the duct is less than that observed at low flow rates. Accordingly, the concentration of Cl at high flow rates is high (and similar to the high-plasma-like acinar levels) (16). When flow is low and time for reabsorption prolonged, [Cl] in the final saliva is low. Amylase concentration also appears to depend on the volume of fluid secreted. However, since the volume of fluid dilutes the amylase, the concentration of amylase decreases as flow rate increases. This assumes that a constant amount of amylase is released at the acinus (17), but definitive conclusions await analysis of glandular amylase levels and further analysis of the role of specific autonomic receptors in regulation of amylase release.

Finally, it has been stated by other investigators that since parasympathetic stimulation elicits greater volumes of saliva, and induces resynthesis of amylase, the total amount secreted must be much greater than that found with sympathetic stimulation (18, 19). Pres-

Table II. Effect of Varying Frequency of Stimulation to Sympathetic Nerve on Norepinephrine Concentration of Rat Parotid Gland after 35 Min of Stimulation of Sympathetic Alone or Combined with Parasympathetic Stimulation[a]

Frequency (Hz)		No. of rats	Norepinephrine concentration (ng/g)		
Sympathetic	Parasympathetic		Stimulated	Control[b]	% Decrease from control
2	16	4	924 ± 142	1210 ± 170	24
5	16	4	675 ± 101	906 ± 80	25
16	16	8	624 ± 143	1152 ± 160	46[c]
2	0	4	549 ± 173	853 ± 46	36
5	0	4	553 ± 208	753 ± 258	27
16	0	5	850 ± 336	1289 ± 364	34

[a] Values are means ± SE.
[b] The controls are the values from contralateral glands.
[c] Value differs significantly from control ($P < 0.01$).

ent data refute this, and show that at all frequencies of sympathetic stimulation, the total amount of amylase secreted in 35 min was greater than that seen with parasympathetic stimulation. Moreover, when sympathetic stimulation was combined with parasympathetic stimulation, the total amount of amylase secreted was, at all frequencies of sympathetic stimulation, two to eight times higher than levels observed with either nerve alone. The high volume with parasympathetic stimulation combined with the high amylase levels observed at all levels of sympathetic stimulation account for this.

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