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Nerve-Stimulated Secretion of Calcium by Rat Submandibular Gland (41661)

JIA-HUEY YU AND CHARLOTTE A. SCHNEYER

Department of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, Alabama 35294

Abstract. The comparative effects of electrical stimulation of parasympathetic (chorda tympani nerve) and sympathetic innervation to the rat submandibular gland on calcium secretion were examined. The separate roles of α- and β-adrenergic receptors in the regulation of calcium secretion during sympathetic nerve stimulation were also determined. The present study shows that the parasympathetic and sympathetic innervations to rat submandibular gland have very different effects on [Ca] of saliva; the regulatory influence of the sympathetic is more prominent than that of the parasympathetic innervation; [Ca] of submandibular saliva evoked by chorda stimulation was about 25 times less than that of saliva evoked by sympathetic stimulation. However, since total volume of chorda-evoked submandibular saliva was about 20 times greater than that of sympathetically evoked saliva, the total output of calcium following stimulation of either autonomic branch was similar. Glandular depletion of calcium for both kinds of nerve stimulation was also similar. Comparison with previous studies on the rat parotid gland shows that the role of the parasympathetic innervation in regulation of [Ca] of saliva and glandular depletion of calcium differed in the two glands while that of the sympathetic innervation was generally similar for both submandibular and parotid glands. Calcium secretion from rat submandibular gland was decreased during sympathetic nerve stimulation in the presence of propranolol, a β-adrenergic blocker, while it was greatly increased when the sympathetic nerve was stimulated in the presence of phentolamine, an α-adrenergic blocker. Thus, it was concluded that with both glands β-adrenoceptors play the major role in the regulation of calcium secretion.

Stimulation of either the parasympathetic or sympathetic innervation to rat parotid gland evokes saliva with high concentrations of calcium (1–3); however, effects of nerve stimulation on calcium secretion from rat submandibular gland have not been studied, although there are some reports describing effects of autonomic agonists (4, 5). Studies on calcium secretion by nerve-stimulated submandibular gland of rat are especially pertinent since results obtained with stimulation of the parasympathetic nerve to the parotid (2) were in sharp contrast to values previously reported for levels of calcium in parasympathomimetically evoked submandibular saliva of rat (4). Moreover, rat submandibular gland, like parotid gland, contains an unusually high concentration of calcium. Thus, the present study was undertaken to examine the comparative effects of electrical stimulation of the parasympathetic and sympathetic innervation to rat submandibular gland on calcium secretion. The separate role of α- and β-adrenergic receptors in regulation of calcium secretion from this gland was also examined by using direct electrical stimulation of the sympathetic nerve in conjunction with the selective adrenergic receptor antagonists.

Materials and Methods. Male Long-Evans rats (4–6 months old, 350–400 g) were used in these experiments. They were maintained on lab chow and water *ad lib* until 18 hr before experimentation when food but not water was removed. Rats were anesthetized with sodium pentobarbital (50 mg/kg body wt) intraperitoneally and the trachea was cannulated to avoid the possibility of respiratory complications. The oral opening of one submandibular duct was cannulated using fine polyethylene tubing (Clay-Adams PE 10). Electrical stimulation of either the sympathetic (cervical sympathetic trunk) or the parasympathetic branch (chorda tympani nerve) of the autonomic innervation to the glands was performed as previously described (3, 6). The nerve was stimulated supramaximally by square wave-pulses (5 msec duration) delivered from a Grass Instruments stimulator at 20 Hz and 4 V (6). To delineate the effects of α- and β-adrenergic responses, the α-adrenergic antagonist, phentolamine (5 mg/kg body wt) (when predominantly β-adrenoceptors are activated), or the β-adrenergic antagonists, propranolol (3 mg/kg body wt) (when predominantly α-adrenoceptors are stimulated) was administered intraperitone-

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ally 20 min prior to initiation of electrical stimulation of the sympathetic nerve. The saliva was collected from the oral end of the submandibular duct cannula using disposable pipets (10–20 μl). Collection periods for saliva were timed and glands weighed, flow rate as well as volume of saliva could be determined. Saliva samples were continuously collected for the period of 60 min of continuous nerve stimulation so that the total output of saliva and calcium could be determined. At the end of each experiment, the stimulated as well as unstimulated gland of the contralateral side were removed rapidly, separated from the adherent sublingual and weighed. Both stimulated and control glands were placed in crucibles and dry-ashed (550°C, 24 hr). The ash was dissolved in 0.5 ml of 1 *N* HCl and thoroughly mixed. Calcium concentrations of saliva and ash samples were determined by titration of the fluorescent calcium–calcein complex with EGTA using an ultramicro automatic calcium titrator (Precision Systems).

Results. The data in Fig. 1 show the course of changes in [Ca] of rat submandibular saliva during a 60-min period of electrical stimulation of the sympathetic or parasympathetic (chorda tympani) innervation to the gland. The [Ca] of chorda-evoked saliva was initially very low (0.3 meq/liter), and remained at these low levels throughout 60 min of stimulation. On the other hand, [Ca] of saliva evoked by stimulation of the sympathetic preganglionic fibers alone was very high (7.5–8.5 meq/liter) during the first 20 min of stimulation; a drop occurred thereafter, and by 50–60 min, [Ca] was 3 meq/liter (Fig. 1). However, the pattern of calcium secretion evoked by sympathetic nerve stimulation was changed by prior administration of phentolamine or propranolol (Fig. 1). For example, the initial [Ca] in the saliva evoked by sympathetic nerve stimulation in the presence of phentolamine (5 mg/kg, ip) was 9–11 meq/liter for the first 20 min; [Ca] then rose to 14 meq/liter, a level that was maintained for the next 20 min; by 50–60 min of nerve stimulation, however, [Ca] was again 10–11 meq/l (Fig. 1). When the [Ca]s of saliva evoked by sympathetic nerve stimulation in the presence of phentolamine were compared with those observed with sympathetic nerve stimulation alone after 20, 30, 40, 50, and 55 min, differences between the two were statistically significant ($P < 0.001$).

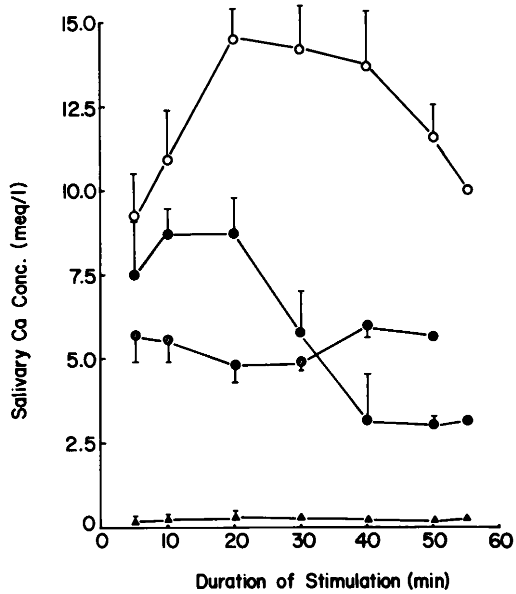

FIG. 1. Effects of stimulation of the parasympathetic nerve and sympathetic nerve alone or in the presence of phentolamine (α-adrenergic antagonist) or propranolol (β-adrenergic antagonist) on changes in calcium of submandibular saliva with time. Phentolamine (5 mg/kg body wt) and propranolol (3 mg/kg body wt) were administered intraperitoneally 20 min before the initiation of sympathetic nerve stimulation, which was continued for 60 min. Neither phentolamine nor propranolol themselves induced salivary flow. (▲) parasympathetic nerve (chorda) stimulation; (●) sympathetic nerve stimulation alone; (○) sympathetic nerve stimulation in the presence of phentolamine; (◍) sympathetic nerve stimulation in the presence of propranolol. Values represent means ± SE of at least three observations.

The [Ca] of saliva evoked by sympathetic nerve stimulation in the presence of propranolol (3 mg/kg, ip) differed significantly from levels observed with sympathetic nerve stimulation alone or in combination with phentolamine (Fig. 1). Initial [Ca] of saliva evoked under this condition of stimulation was 5.5 meq/liter and it remained at this level throughout the 60 min of stimulation (Fig. 1).

Stimulation of the chorda tympani nerve to rat submandibular gland evoked saliva with very high flow initially and flow remained high throughout the 60-min period of nerve stimulation. Thus, total volume of secretion evoked by chorda stimulation was 20 times higher than that obtained with sympathetic nerve alone (Table I). However, there was no statistically significant difference between total volume of saliva obtained with stimulation of the sympathetic nerve alone and that observed with sympathetic nerve stimulation in the presence of either phentolamine or propranolol (Table I).

Total calcium output of chorda-evoked saliva from submandibular gland (60 min) was approximately 520 neq and similar to the value observed with stimulation of the sympathetic nerve alone (Table I). Total calcium output with stimulation of the sympathetic nerve in the presence of phentolamine was, however, higher than that obtained with either sympathetic nerve stimulation alone or sympathetic nerve stimulation in the presence of propranolol (Table I). [Ca]s of the submandibular gland after a 60-min period of stimulation of the parasympathetic or sympathetic nerve alone were greatly reduced (Table I). However, glandular depletion of calcium with stimulation of the sympathetic nerve was somewhat greater than that observed with stimulation of the parasympathetic nerve (Table I). Glandular depletion of calcium with stimulation of the sympathetic nerve in the presence of propranolol was less than that obtained with the sympathetic nerve alone ($P < 0.05$).

Discussion. The [Ca] of saliva induced by electrical stimulation of the parasympathetic innervation to submandibular gland of rat resembled the low level reported for parasympathomimetically stimulated saliva (4); these differed markedly, however, from the unusually high levels (10–12 meq/liter) found in saliva evoked by stimulation of the parasympathetic nerve to parotid gland of rat (1, 2). On the other hand, [Ca] of submandibular saliva with stimulation of the sympathetic innervation to the gland was high, and resembled that of sympathomimetically stimulated saliva (5); it also closely resembled that of parotid saliva evoked by stimulation of the sympathetic nerve (3). Thus, it is clear that the role of the parasympathetic innervation in regulation of [Ca] of the saliva is not the same for the two glands, while that of the sympathetic innervation is more similar (2, 4).

The parasympathetic and sympathetic innervations to submandibular gland have very different effects on [Ca] of saliva; the regulatory influence of the parasympathetic is less prominent than that of the sympathetic innervation. The [Ca] of submandibular saliva with chorda stimulation was about 25 times less than that of saliva with sympathetic stimulation. However, since total volume of chorda-evoked saliva was 20 times greater than that of sympathetically evoked saliva, the total output of calcium following stimulation of either autonomic branch was similar. Glandular depletion of calcium for both kinds of nerve stimulation was also similar. Thus, it appears that there was good correlation between the amount of calcium output into saliva and the degree of glandular calcium depletion of submandibular gland with both types of autonomic nerve stimulation.

In contrast to these findings on submandibular gland, with parotid, the regulatory role

TABLE I. TOTAL OUTPUT OF CALCIUM AND VOLUME IN RAT SUBMANDIBULAR SALIVA FOLLOWING STIMULATION OF THE PARASYMPATHETIC OR SYMPATHETIC INNERVATION

Kind of stimulation	Mean flow rate (μl/min/g wet wt)	Saliva: Total volume (μl)	Saliva: Total Ca output (neq)	Gland Ca depletion (%)
PARA	223	2626 ± 317 (5)	517 ± 122 (5)	41 ± 2 (3)
SYM	21	119 ± 15 (4)	696 ± 159 (4)	52 ± 6 (4)
PROP + SYM	12	80 ± 18 (5)	406 ± 95 (5)	37 ± 3 (5)‡
PHENTO + SYM	8	75 ± 10 (5)*	957 ± 182 (5)†	59 ± 11 (4)

Note. Values are means ± SE. Numbers in parentheses refer to numbers of rats. PARA = chorda tympani nerve; SYM = sympathetic nerve stimulation alone; PROP + SYM = sympathetic nerve stimulation in the presence of propranolol; PHENTO + SYM = sympathetic nerve stimulation in the presence of phentolamine. Propranolol (3 mg/kg body wt, ip) or phentolamine (5 mg/kg body wt, ip) was injected 20 min before nerve was stimulated. *$P < 0.05$ as compared to value obtained with SYM alone; †$P < 0.05$ as compared to value obtained with PROP + SYM; ‡$P < 0.05$ as compared to value obtained wth SYM alone. Total calcium output for the whole 60-min stimulation period was calculated as sum of volume of saliva collected for each time interval (μl) × calcium concentration (neq/μl).

of both autonomic innervations on [Ca] of nerve-evoked saliva is very similar (2). Stimulation of either the sympathetic or parasympathetic nerve to rat parotid produced saliva with a similarly high [Ca] (1–3). However, since total volume of parotid saliva secreted over 60 min is 10 times higher with parasympathetic than with sympathetic stimulation, the total output of calcium of parasympathetically evoked saliva is also 10 times higher than that of sympathetically evoked saliva (2). Surprisingly, there is virtually no glandular depletion of calcium with parasympathetic stimulation of parotid (2, 3), whereas a marked depletion was found with sympathetic nerve stimulation (3). Thus, it is clear that estimates of neural regulation of calcium secretion based on amount of its glandular depletion may not always be reliable. It would be erroneous to conclude that the parasympathetic innervation has little role in regulation of calcium secretion of the parotid. In fact, the high concentration of calcium in the parotid saliva and the large output of calcium over the 60 min of stimulation of the parasympathetic innervation suggest that parasympathetic nerve activity regulates not only secretion of calcium into saliva but also uptake of calcium into parotid gland. This was partly confirmed by the experiments using ^{45}Ca uptake into parotid gland (7). However, present data show that the parasympathetic innervation probably does not regulate calcium uptake in submandibular gland since the output of calcium into parasympathetic saliva is paralleled by emptying of calcium from cells of the gland.

The role of the parasympathetic innervation in regulation of calcium secretion is thus different for the two glands. That of the sympathetic is also not identical for the two glands although there are some similarities. Thus, the depletion of glandular calcium with 60 min of stimulation of the sympathetic nerve is somewhat less for parotid than for submandibular (27 as compared to 40%), and the time course of calcium release into saliva is, for the last 40 min, also very different for the two glands, with calcium falling markedly after 20 min of sympathetic stimulation of the submandibular but remaining elevated for the 60 min of such stimulation of the parotid (3). The roles of α and β-adrenoceptors activated during stimulation of the sympathetic nerve are generally similar for parotid and submandibular gland. The present data show that when β-adrenoceptors are stimulated as a consequence of stimulation of the sympathetic innervation to the submandibular gland in the presence of phentolamine, high calcium levels in the saliva are similar to those found with isoproterenol stimulation (5). The lowest levels of calcium are found when α-adrenoceptors are activated during stimulation of the sympathetic innervation in the presence of propranolol. These differences can also be seen by examination of data on total output of calcium and depletion of glandular calcium. However, with parotid gland these differences are always evident (3), but with submandibular gland statistically significant differences are not recorded for all parameters. Nevertheless, it is clear that [Ca] of saliva appears to be a useful parameter for assessment of the kind of receptor activated during sympathetic nerve stimulation.

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