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Neural Regulation of Calcium and Amylase of Rat Parotid Saliva[1] (39891)

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Introduction. Stimulation of rat parotid gland by catecholamines, *in vitro*, results in secretion of large amounts of calcium and amylase, with the two moieties being secreted in parallel (1). Since calcium and amylase are secreted in parallel, it is believed that the two are "packaged" together in microsomes and then secreted together across the luminal cell membranes (1). It is not known, however, if such a parallelism exists with all kinds of stimulation. The present work was undertaken to examine, *in vivo,* the relationship between secretion of amylase and of calcium with diverse kinds of stimulation. These included measurement, for the first time, of calcium levels of saliva obtained by direct stimulation of the parasympathetic and sympathetic pathways to the parotid. Amylase activity of saliva samples concurrently collected was also determined. For purposes of comparison with available *in vitro* data, isoproterenol and pilocarpine were also used.

Materials and methods. Male Long-Evans rats 4–6 months of age 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. Just prior to experimentation, rats were anesthetized by ip administration of sodium pentobarbital in doses of 50 mg/kg of body weight. Following anesthetization, the trachea was cannulated to avoid the possibility of respiratory complications. Collection of saliva samples was performed as previously described (2). For stimulation of flow, the autonomic agents pilocarpine (5 mg/kg) or isoproterenol (16 mg/kg) were injected ip, or electrical stimulation of either sympathetic (superior cervical ganglion) or parasympathetic branch (auriculotemporal nerve) of the autonomic innervation to the glands was used. A Grass stimulator, SD5, delivering square-wave pulses of 4 V at a frequency of 20 pulses/sec and duration of 5 msec, was used (3, 4). Flow rate was determined by measuring the time required for collection of a given volume of saliva and relating this to weight of the gland. Stimulation was continuous and collection of samples was continuous so that the total output of saliva and its components could be measured. Calcium concentration was determined on saliva samples by titration of the fluorescent calcium–calcein complex with EGTA (Fiske automatic calcium titrator). Atomic absorption analysis of calcium was also done and results were compared with those obtained with the titration method; agreement between the two was 100%.

Amylase activity of appropriately diluted samples of saliva was determined by the method of Myers *et al.* (5). Amylase activity was expressed as milligrams of glucose formed per milligram of saliva in a 15-min digestion period at 37°. Samples of saliva were obtained at 5-min intervals following initiation of stimulation; stimulation was maintained and collections were made for as long as 90 min in most experiments.

Results. The data in Fig. 1 show the time course of calcium secretion from rat parotid with various types of autonomic stimulation. With supramaximal stimulation of the auriculotemporal nerve, calcium levels were initially high (11.9 ± 1.0 mequiv/liter) and remained at this level for the first 20 min of continuous stimulation. A decrease that was statistically significant ($P < 0.05$) was not evident until 40 min after initiation of stimulation, when calcium levels dropped to about 9.7 mequiv/liter; the value was not, at 90 min, significantly different ($P > 0.05$) from the 40-min level, but it represented an overall change of 29% from initial levels.

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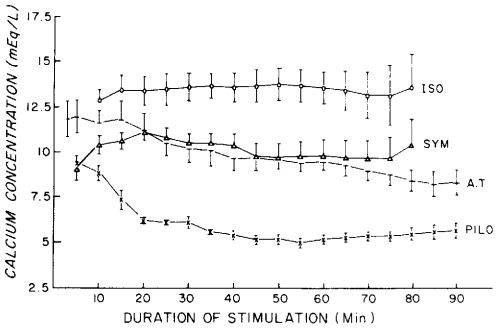

FIG. 1. Changes in calcium concentration of parotid saliva during prolonged stimulation by various modes (ISO., isoproterenol; SYM., sympathetic nerve; A.T., auriculotemporal nerve; PILO., pilocarpine). Duration of stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.

Concentration of calcium of saliva evoked by supramaximal stimulation of the sympathetic innervation to the parotid was approximately 9 mequiv/liter initially, and thus was somewhat lower than initial levels obtained with parasympathetic nerve stimulation (Fig. 1). However, within the next 5 min a significant increase occurred and a maximal level of 11.1 ± 0.4 mequiv/liter appeared at 20 min. For the duration of stimulation (the ensuing 60 min) there were no statistically significant changes and calcium concentration at 20 and 80 min was approximately 10 mequiv/liter (Fig. 1). As a consequence of the significant increase between 3 and 20 min, the course of calcium secretion with sympathetic nerve stimulation was, except for this initial phase, similar to that observed with stimulation of the auriculotemporal nerve. Furthermore, even after 80 min of stimulation, the terminal calcium levels with sympathetic nerve stimulation were not statistically different ($P < 0.05$) from those recorded with stimulation of the auriculotemporal nerve (Fig. 1).

Since catecholamines (epinephrine and isoproterenol) and the parasympathomimetic agent, pilocarpine were used *in vitro* to stimulate secretion from parotid slices, comparisons between effects of nerve and chemical stimulation were compared *in vivo*. Initial calcium levels of saliva evoked only 10 min after ip injection of isoproterenol exceeded 12.8 ± 0.6 mequiv/liter, showed an immediate increase to 13.4 ± 0.8 mequiv/liter, and remained at this high level for the entire 80 min of stimulation.

With pilocarpine, on the other hand, initial calcium levels were only 9.4 ± 0.3 mequiv/liter and were thus significantly less ($P < 0.025$) than those observed with parasympathetic nerve stimulation (Fig. 1). Furthermore, a considerable drop occurred within the first 15–20 min to 6.2 ± 0.7 mequiv/liter; the lowest levels, 5 mequiv/liter were reached and maintained thereafter.

The time course of amylase secretion in response to diverse autonomic stimuli is depicted by the data in Fig. 2. With supramaximal stimulation of the auriculotemporal nerve, the evoked saliva was very low in amylase activity; in fact, initially and for the entire 90 min, amylase activity was 18.5 ± 2.5 mg of reducing substance/mg of saliva.

With sympathetic nerve stimulation, on the other hand, amylase activity was initially very high (454 ± 33 mg/mg), and with continuation of stimulation, a significant increase in amylase activity to maximal levels of 575 mg/mg was recorded 15–20 min after initiation of stimulation. A decrease occurred thereafter with a plateau between 450 and 515 mg/mg maintained for the subsequent 30 min.

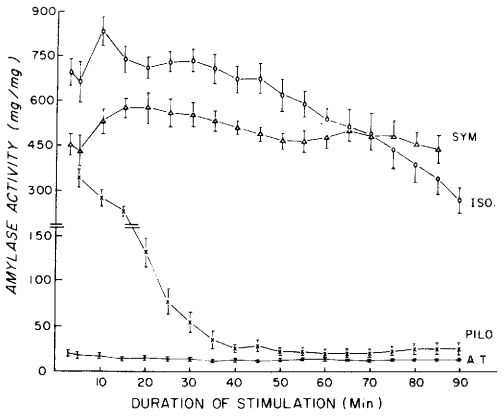

FIG. 2. Time course of change in amylase activity of parotid saliva in response to various modes of stimulation (ISO., isoproterenol; SYM., sympathetic nerve; PILO., pilocarpine; A.T., auriculotemporal nerve). Duration of stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.

Isoproterenol stimulation caused even higher levels of amylase activity than those observed with sympathetic nerve stimulation. Initial levels were 662 ± 66 mg/mg, a statistically significant increase ($P < 0.05$) to 834 ± 46 mg/mg occurred within the first 5 minutes, and for the next 35 min the levels remained at about 700 mg/mg. There was only a gradual decrease with time, and after 90 min, levels were as high as 269 ± 42 mg/mg.

Pilocarpine, while evoking saliva with initial levels 20–30 times greater than those observed with auriculotemporal nerve stimulation, caused activity levels significantly lower than those observed with either isoproterenol or sympathetic nerve stimulation (Fig. 2).

The course of change in amylase activity closely paralleled that of calcium concentration when sympathetic nerve stimulation, or pilocarpine, was employed. However, this was not the case with stimulation involving either the auriculotemporal nerve or administration of isoproterenol. With auriculotemporal stimulation amylase levels were low (about 18 mg/mg), initially and throughout the 90-min period of stimulation, but calcium levels were high (11 initially) and decreased at a slow rate thereafter. Thus, no parallelism in secretion of these two moieties was apparent either in the course of change or in the relative values. With isoproterenol stimulation, on the other hand, while amylase levels and calcium levels were initially (within first 10 min) high, amylase levels thereafter decreased, but calcium levels did not. Thus, in this case also, the course of change did not show a parallelism in secretion of the two moieties.

Finally, it is apparent that while flow rate could be an important factor in regulation of calcium levels, this was obviously not the case here (Fig. 3). First, although flow rates with parasympathetic nerve stimulation were very high (91 ± 1.3 μl/min/g of gland) and those with sympathetic nerve stimulation were very low (6.5 ± 1.4 μl/min/g), calcium concentrations were very similar throughout the 90 min, with both being very high in the first 5-min interval and decreasing only moderately with time. Second, it is clear that flow rates with either parasympathetic nerve stimulation or pilocarpine stimulation were not constant and both decreased markedly within the first 15–20 min after initiation of stimulation. These changes in flow rate were accompanied by marked decreases in amylase and calcium concentration when pilocarpine stimulation was used, but with stimulation of the parasympathetic nerve amylase concentration did not change at all and calcium increased somewhat during this interval of marked change in flow rate.

Discussion. Secretion of amylase is dependent on the kind of autonomic stimulation used to cause secretory activity. Adrenergic stimulation, especially that involving β receptors, causes a marked secretion of amylase whereas cholinergic stimulation causes only a very modest secretion of this enzyme (6, 7). These differences in amylase secretion exist when either autonomic drugs or direct nerve stimulation is used to evoke saliva.

Secretion of calcium was also believed to be dependent on the kind of autonomic stimulation used to evoke secretion. Present data on whole parotid as well as earlier reports on whole submaxillary (8–10) or parotid slices (10) indicate that adrenergic agents cause a more marked secretion of calcium than do parasympathomimetic agents, such as pilocarpine. However, the influence of direct stimulation of the neural pathways to the parotid on secretion of cal-

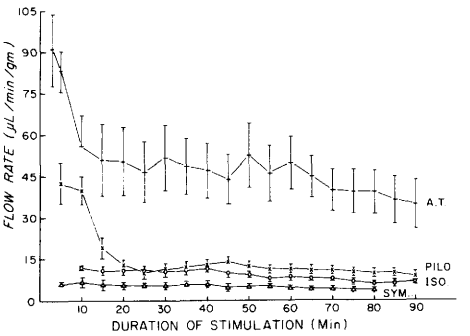

FIG. 3. Time course of change in flow rate of parotid saliva in response to various modes of stimulation (ISO., isoproterenol; SYM., sympathetic nerve; PILO., pilocarpine; A.T., auriculotemporal nerve). Duration of stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.

cium had not previously been examined; present findings show that these effects were unexpectedly different from those produced by autonomic agents. Thus, calcium concentration of saliva evoked by stimulation of the parasympathetic nerve to parotid were as high as those of saliva evoked by stimulation of the sympathetic nerve. In fact, they were very similar in magnitude to values of saliva evoked by isoproterenol, the most potent of the adrenergic agents in inducing secretion of calcium and amylase. Thus, kind of nerve stimulation did not influence levels of calcium in saliva. Since, on the other hand, amylase levels were influenced by kind of autonomic stimulation (whether neural or drug), a marked difference exists between neural control of amylase secretion and neural control of calcium secretion.

The patterns of amylase and calcium secretion were accordingly different also. With stimulation of the cholinergic innervation, there was a conspicuous lack of parallelism in secretion of calcium and amylase at all times. Thus, calcium concentration was high initially, but as flow rate decreased with time, calcium concentration also decreased, and a 30% decrease was ultimately evident after 90 min of stimulation. Amylase concentration, on the other hand, was very low initially and remained unchanged throughout the 90 minutes. Such data do not support the idea of "packaging" of the two moieties (1) when cholinergic nerve stimulation is employed. Even with isoproterenol stimulation, while both amylase and calcium levels were high initially, that of amylase decreased conspicuously with time whereas that of calcium did not. The most convincing evidence of a parallelism between secretion of amylase and calcium was that obtained with either pilocarpine stimulation or stimulation of the sympathetic nerve. The present work thus suggests that the pathways for secretion of calcium and of amylase may be different. Earlier work showed that calcium and sialic acid, for example, were not secreted in parallel from rat submaxillary gland whereas total protein and calcium secretion did show a parallelism (9). Calcium secretion may occur by more than one route: one involving a packaging of the calcium with the secretable protein (11), and another, in addition to this one, involving addition to the salivary fluid without binding to protein or packaging with proteins.

Summary. The course of amylase and calcium secretion into saliva is described under diverse conditions of autonomic stimulation of parotid. Of special importance is the fact that for the first time calcium levels of saliva were measured under conditions of nerve stimulation. Initial levels of calcium with stimulation of the parasympathetic innervation were as high as those evoked by stimulation of the sympathetic innervation. While calcium and amylase levels were characteristically high with adrenergic stimulation (either following drug administration or nerve stimulation), amylase levels following stimulation of the parasympathetic nerve were characteristically low. In fact, a parallelism between secretion of calcium and amylase was not observed under conditions of cholinergic nerve stimulation, but was observed with adrenergic nerve stimulation. It is suggested that with cholinergic nerve stimulation mediation of calcium and amylase secretion may differ from that observed with adrenergic stimulation.

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