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# Role of Sympathetic Pathway in Secretory Activity Induced in Rat Parotid by Feeding[1] (38333)

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Direct stimulation of either the cholinergic or the adrenergic secretomotor pathway to salivary glands causes flow of saliva, but the flow is characteristically sparse with adrenergic stimulation and high with cholinergic stimulation (1). In fact, these differences in flow in response to direct stimulation have to a large extent been responsible for the view that the cholinergic pathway is the physiologically important one. This may indeed be the case if rate of flow alone is used as a criterion of secretion. It may not be true if secretion of protein rather than fluid is used as a criterion. Thus, recent work has shown that adrenergic stimulation, especially that involving a prominent action on $\beta$ receptors, has a pronounced effect on protein secretion by the glands, with secretion of protein reaching very high levels after such stimulation (2–4). On the other hand, with cholinergic stimulation, protein levels of saliva are characteristically low (5). Thus, it seems reasonable to postulate that, in normal secretion, both branches have stimulatory roles but that these roles are different: *i.e.*, a principal role of the adrenergic innervation is to cause secretion of gland proteins, with elaboration of water only a minor role; a primary role of the cholinergic innervation is to cause elaboration of copious quantities of fluid. Evidence for these separate roles of the two branches in normal salivary gland activity has been sparse, especially with regard to the role of the sympathetics (1, 6). Therefore, the principal aim of the present work was to assess the role of each autonomic branch under physiological conditions, *i.e.*, during normal eating. However, the usually employed criteria of grandular secretory activity are based on amount and/or composition of saliva produced; during masticatory activity, such measurements are difficult; therefore, the degree of depletion of gland amylase was used as the criterion of secretion.

*Materials and Methods.* Six-mo-old Long–Evans female rats were maintained on lab chow, and water, *ad lib.* On the day before the experiment, food was removed at 5 PM, and the next morning, between 8 and 10 AM, rats were subjected to the experimental procedures. This routine was followed so that maximal levels of amylase were established in the parotid gland. The experimental procedures involved stimulation of the parotid gland by various modes: supramaximal (5) electrical stimulation of the auriculotemporal nerve or superior cervical ganglion (20 pulses/sec, and 3 V, Grass SD 5 Stimulator); permitting animals to eat either solid or liquid food; or administration of supramaximal (5) doses of pilocarpine (2 mg/rat, ip). In some cases, the period of stimulation was preceded by acute ipsilateral removal of a superior cervical ganglion, or by administration of cholinergic (atropine) or adrenergic (propranolol and dibenzyline) blocking agents. These agents were administered ip in doses of 0.25–2 mg/rat 20 min before refeeding with chow. During the period of eating, 6 g or 6 ml of food were usually consumed per rat. After the hour of feeding, animals were anesthetized with 1% Nembutal, and the parotid glands were rapidly removed and weighed on a torsion balance. The gland was then divided into weighed aliquots so that amylase and dry weight could be determined for each gland. Dry weight was measured after 24 hr at 105°. Amylase was determined on appropriately diluted supernatant of gland homogenate, using the method of Myers, Free, and Rosinski (7).

*Results.* The data in Table I show that amylase activity of rat parotid gland is decreased after

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TABLE I. Effects of Different Conditions of Stimulation on Amylase Activity and Water Content of Rat Parotid Gland.

| Group | No of rats | Kind of stimulation | Conditions of stimulation | Parotid gland | | Decrease in amylase (%) |
|---|---|---|---|---|---|---|
| | | | | $H_2O$ content (%) | Amylase activity (mg/mg wet wt.)[a] | |
| 1 | 20 | None (control) | — | 72 ± 0.5 | 525 ± 13 | — |
| 2 | 3 | Auriculotemporal nerve | Intermittent, supramax. electr., 1 hr | — | 520 ± 23 | 0 |
| 3 | 9 | Auriculotemporal nerve | Continuous, supramax. electr., 1 hr | 72 ± 1.0 | 408 ± 29 | 22[b] |
| 4 | 4 | Superior cervical ganglion | Continuous, supramax. electr., 1 hr | 70 ± 0.7 | 308 ± 52 | 41[b] |
| 5 | 6 | Pilocarpine | 2 mg ip, .5 hr duration | — | 288 ± 20 | 45[b] |
| 6 | 15 | Pilocarpine | 2 mg ip, 1 hr duration | 74 ± 1.0 | 275 ± 19 | 48[b] |
| 7 | 8 | Pilocarpine | 2 hr duration | 73 ± 1.2 | 286 ± 30 | 46[b] |
| 8 | 6 | Pilocarpine | After sympathectomy, 2 mg ip, 1 hr duration | 73 ± 0.7 | 512 ± 24 | 0 |
| 9 | 15 | Chow feeding | 1 hr duration[c] | 73 ± 0.5 | 389 ± 13 | 26[b] |
| 10 | 10 | Chow feeding after atropine | 1 hr duration,[c] after .25 mg atropine | 73 ± 1.5 | 392 ± 24 | 25[b] |
| 11 | 8 | Chow eating, after propranolol + dibenzyline | 1 hr duration,[c] after 2 mg propr. and 1 mg dibenz. | 69 ± 0.7 | 528 ± 13 | 0 |
| 12 | 7 | Liquid diet eating | 1 hr duration[c] | 72 ± 0.5 | 517 ± 22 | 0 |

Values are means ± SE.

[a] Expressed as milligrams reducing substance formed per milligrams wet tissue in 15 min at 37°.

[b] Diff. from control is significant ($P < 0.01$). $H_2O$ did not differ from Control ($P > 0.01$) for any group.

[c] Rats in these groups each consumed approx 6 g of chow, or 6 ml liquid diet in the feeding period.

autonomic stimulation, and that the decrease is related to the kind and duration of such stimulation. When the postganglionic parasympathetic fibers to the parotid were intermittently stimulated, over a period of 1 hr with supramaximal electrical shocks, gland amylase was not perceptibly reduced (Table I, Group 2); even when stimulation of the nerve was continued without interruption for an hour, gland amylase was reduced by only 22% (Table I, Group 3). When supramaximal electrical stimulation of the sympathetic innervation was employed, amylase levels after 1 hr of continuous stimulation were reduced by approximately 40% (Table I, Group 4). An additional method involving stimulation of the sympathetic pathway was also used. Pilocarpine acts not only on the muscarinic receptors of the gland but also causes stimulation of the sympathetic innervation of the gland by an action mediated through the superior cervical ganglion (9–11). Since this action on the neurally intact gland caused combined cholinergic and adrenergic effects, a separation of these two influences was effected by acute ipsilateral removal of a superior cervical ganglion prior to administration of the pilocarpine (11). Thus, it is clear from the data in Table I, (Group 6) that an hour after pilocarpine administration, amylase was reduced nearly 50%, in the neurally intact parotid, but was not at all reduced in the acutely

sympathectomized contralateral mates (Table I, Group 8). The effects of pilocarpine on the neurally intact gland were in fact evident even 30 min after administration of the pilocarpine (Table I, Group 5) and were not more pronounced when measurements were made 2 hr after drug administration (Table I, Group 7).

The data in Table I also show the relative effectiveness of stimulation induced by eating on amylase levels of rat parotid glands. When amylase activity of parotid was measured 1 hr after fasted rats were refed with solid chow, a 26% reduction in concentration of this enzyme was found (Table I, Group 9). If liquid, instead of solid, food was given, no reduction in amylase levels was effected (Table I, Group 12). Thus, eating solid food caused stimulation of the gland that was not induced by consuming liquid food, and it was, therefore, assumed that autonomic reflexes were extensively involved in the former but not in the latter situation (12, 13). To determine the extent of such autonomic involvement in the amylase depletion induced by eating solid food, the reduction caused by chow feeding alone was compared with the reduction induced by chow feeding that followed administration of specific autonomic antagonists.

When dibenzyline and propranolol were administered at the same time 20 min prior to introduction of food to the fasted rats, amylase activity of the parotid (measured 1 hr later) was not reduced by feeding, indicating that amylase depletion was completely prevented by these agents (Table I, Group 11). Administration of atropine 20 min prior to feeding did not inhibit gland emptying of amylase stores during feeding (Table I, Group 10); thus, the reduction induced by feeding in the presence of atropine was the same as that induced by feeding alone (25%).

Since glandular activity results in a change in water of the tissue (14), water content of the parotid was determined after each kind of stimulation. It was found that water content did not change by more than a few percent with any kind of stimulation (Table I).

*Discussion.* The data clarify the role of the sympathetic innervation in regulating salivary secretion during normal mastication. Furthermore, this regulatory effect is concerned principally with secretion of proteins. It has been difficult to assess the role of the sympathetic innervation during normal physiological activity of the gland, although from other data, stimulation of adrenergic receptors causes a marked secretion of protein from the gland, either into saliva (2, 3, 11), blood (8), or *in vitro,* into medium (15). The present data show that the stimulation effected by eating solid food can rapidly cause significant reduction in gland amylase (14) and that the autonomic influence involved in mediating this effect is sympathetic. Thus, administration of atropine in doses that block parasympathetic nerve response (16) did not prevent the depletion induced by eating. On the other hand, activity mediated through cholinergic receptors under physiological conditions of stimulation, did not cause depletion of gland amylase, since the prior administration of $\alpha$ and $\beta$ adrenergic antagonists before feeding prevented the chow-induced depletion.

While physiological conditions of gland stimulation are provided when masticatory activity accompanies eating, under certain circumstances, such as consumption of liquid diet, masticatory activity is diminished or even absent (12, 13). In this case, there is virtual elimination of neurally mediated activity of the gland (13) and, therefore, gland amylase is not reduced. Although kind of diet influences the amount and composition of secretion (6), recently differences in effects induced by solid and liquid diet have also been recognized (12).

The data also establish the validity of using degree of depletion of gland amylase to indicate kind and duration of autonomic stimulation. Thus, cholinergic stimulation characteristically induced little depletion of gland amylase, whether the cholinergic innervation was stimulated reflexly, or directly, by electrical stimulation, or stimulation of the gland by injection of parasympathomimetic drugs. Adrenergic stimulation, on the other hand, especially that involving $\beta$ receptors (17), effected a marked depletion in gland amylase under all conditions of stimulation. Thus, even though the effects induced by electrical stimulation of the sympathetic innervation were variable, the depletion induced was, on the average, considerable. Reflexly induced stimulation also caused significant reduction. The most convincing evidence of the difference in behavior of the two kinds of receptors was derived from the experiments with pilocarpine. The effects caused by adrenergic glandular receptors can be separated from those produced by cholinergic receptors on the gland simply by comparing pilocarpine-induced amylase deple-

tion in the neurally intact gland with that induced in the acutely sympathectomized gland. Thus, with stimulation involving only an effect on cholinergic receptors of the gland, amylase is not reduced, whereas it is significantly reduced when the sympathetic pathway is concurrently stimulated. The data also implicate $\beta$ adrenergic receptors in the sympathetically induced amylase secretion. Thus, when propranolol is administered prior to injection of pilocarpine, saliva amylase levels are reduced to those found with cholinergic stimulation alone (4). Furthermore, preliminary experiments suggest that propranolol can block the depletion of gland amylase usually induced by pilocarpine and can also prevent the depletion induced with chow feeding. Finally, there is indirect implication of the role of $\beta$ adrenergic receptors in causing amylase reduction. For example, with simultaneous administration of both $\alpha$ and $\beta$ adrenergic blocking agents, the presence of the $\beta$ receptor antagonist may nullify $\alpha$ adrenergic blockade (18). Nonetheless, even though $\alpha$ receptors may then be active, complete inhibition of gland emptying of amylase occurs. Thus, with the autonomically mediated glandular activity induced by eating solid food, a primary role for $\beta$ receptors in secretion of amylase is, therefore, indirectly implicated.

Finally, while cholinergic stimulation is normally not of sufficient duration or intensity to cause a change in gland amylase, cholinergic receptors do have some role in secretion of amylase (5). Thus, amylase appears in saliva evoked by stimulation of the parasympathetic innervation (5), or by injection of parasympathomimetic drugs (3–5); furthermore, when the intensity and duration of cholinergic stimulation are great enough even gland levels may be altered.

*Summary*. A prominent role for the sympathetic innervation in the secretory activity reflexly induced in parotid gland during eating is shown by present data. This role is concerned principally with the secretion of proteins, *e.g.*, amylase. Parotid glands of rats fasted overnight to establish maximal glandular levels of the enzyme were stimulated by eating solid (but not liquid) diet; measurements of amylase activity made 1 hr later showed a 26% decrease in amylase activity of the gland. If atropine, in low dose, was administered just prior to the refeeding, there was no inhibition of the emptying; if propranolol and dibenzyline were administered prior to refeeding, the emptying was inhibited. Thus, the emptying of gland amylase induced by eating solid food is mediated through the sympathetic pathway. Cholinergic stimulation, either by feeding, by direct electrical stimulation of the auriculotemporal nerve, or by injection of a parasympathomimetic agent, produced little alteration of gland levels of enzyme. On the other hand, adrenergic stimulation had a profound effect in causing depletion of gland levels of amylase that was apparent from direct electrical stimulation of the sympathetic innervation, from use of pilocarpine and from experiments with refeeding of solid chow.

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