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Calcium Concentration of Saliva and Salivary Glands of Rat after Cyclocytidine (41758)

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Abstract. Cyclocytidine (CC), in addition to its antitumor properties, also causes copious flow of saliva. Calcium concentration of CC-evoked saliva from submaxillary (SM) and parotid (PA) glands of adult rats was initially 7 meq/liter and 15 meq/liter, respectively, and thus resembled that of sympathetically evoked secretion. From previous data, as well as present data, this is expected since CC apparently causes release of norepinephrine (NE) from adrenergic nerve endings. Present data also confirm that CC causes NE release since a single dose of reserpine (RES) (5 mg/kg), administered 24 hr prior to injection of CC in order to cause depletion of NE prevented the action of CC. Furthermore, the NE released by CC acts principally on β-adrenergic receptors since propranolol administered prior to CC caused a marked reduction in flow and [Ca] of saliva, and prevented the usual CC-induced depletion of glandular calcium. An increase in [Ca] of SM but not PA gland was also caused by chronic (daily injections of 500 mg/kg body wt for 3 days) administration of CC. The same threefold increase was observed 2 days after injection of a single dose of CC also. The increase in glandular calcium was not prevented by propranolol, thus suggesting that this effect of CC on glandular [Ca] was probably not β-mediated. The calcium increase may, however, be the result of depletion of NE. Thus, [Ca] of SM of CC-treated rats, that of RES-treated rats, and that of rats treated with RES + CC were very similar. If the mechanism of action of the two drugs were different (not NE depletion), the combined action of the two would have been additive.

Cyclocytidine (CC), a potent antitumor agent (1), also has a number of undesirable side actions that result from β-adrenergic activity induced by the drug (2, 3). Included in these β-mediated side effects are sialorrhea (3, 4) and hypertrophy of salivary glands and heart (2, 3). CC causes these effects by inducing release of norepinephrine (NE) from nerve endings (3). The β-adrenergic agonist, isoproterenol, also causes sialorrhea and hypertrophy (5, 6) and in addition causes an increase in glandular concentration of calcium (7, and unpublished data) for which there is thus far no explanation. The present investigation was undertaken to see if an increase in glandular [Ca] could be induced by CC, and if so, whether this increase and the hypertrophy were both sequelae to CC-induced activation of β-adrenoceptors. On the other hand, they might be separate events, with increase in [Ca] being non-β-mediated and hypertrophy β-mediated. It is anticipated that the cause, if not the significance, of the calcium increase may be disclosed from present work. Furthermore, since CC causes effects that mimic acute as well as chronic stimulation of the sympathetic nerves to the glands, [Ca] of CC-evoked saliva should resemble that of saliva evoked by stimulation of the sympathetic innervation (8, 9). In addition, reserpine (RES), a drug that causes norepinephrine (NE) depletion (10), will be used in conjunction with CC to provide evidence that CC acts by causing release of NE, and that this is in fact a physiological release of the neurotransmitter NE by CC and not from release of NE from adrenergic vesicles destroyed by CC.

Materials and Methods. Female Long–Evans rats, 4 months old and weighing approximately 200 g, were used in these experiments. Animals were maintained on lab chow and water except for the 18 hr preceding acute experimentation when food but not water was removed. For acute CC administration, single doses of CC (500 mg/kg) were administered intraperitoneally to 6–8 rats; for chronic CC administration, CC was given for 3 days in daily doses of 500 mg/kg. In some rats, a single dose of RES (5 mg/kg) was dissolved in 20% ascorbic acid and administered 24 hr prior to injection of CC. In other rats, propranolol (5- or 20-mg/kg doses) was administered as a single injection or daily for 3 days 20 min prior to administration of CC. For controls, animals were injected with either propranolol, RES, 20% ascorbic acid, or normal saline. Saliva

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elicited by CC alone or in combination with other drugs was collected from the cannulated submaxillary (SM) or cut parotid (PA) ducts by micropipet. Flow rate was determined by measuring the time required for collection of a given volume of saliva. Gland weight was obtained by weighing the organs on a torsion balance. Statistical analysis of data was performed using Student's *t* test.

Calcium concentration of saliva samples was determined using an automatic calcium titrator (Precision Systems). For calcium determination of SM and PA glands, the glands were rapidly removed from rats previously anesthetized with Nembutal (50 mg/kg body wt), weighed rapidly, 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. This solution was used for calcium determination by the calcium titrator as already described.

Results. Salivary flow from SM and PA glands was initiated within 3–5 min after a single injection of CC (500 mg/kg, ip); it was very slow during the first hour, but became copious after 60 min. Data in Fig. 1 show time-related changes in flow rate of CC-evoked SM and PA saliva during the 240 min after injection of CC (typical curves from single rats are presented). Flow rate from SM gland increased to maximal levels of 30 μl/min/g at 120 min; it declined thereafter to reach low levels of 5 μl/min/g at 240 min; flow rate from PA reached a maximum of 17 μl/min/g at 120 min and declined to the same low levels seen with SM by 240 min. Mean [Ca] of saliva collected from SM and PA between 60 and 180 min after injection of CC are shown by data in Figs. 2 and 3. [Ca] of PA saliva at 30 min was 15 meq/liter, reached a level of 23 meq/liter at 45–60 min and declined thereafter to 12 meq/liter by 180 min. [Ca] of SM saliva was, in initial samples, 7 meq/liter and was somewhat lower at 180 min (about 5 meq/liter). [Ca] of both glands was reduced by CC, and 270 min after drug administration, [Ca] of PA showed a reduction of 18.5 ± 8.2% (three rats) and [Ca] of SM gland showed a reduction of 49.4 ± 4.8% (three rats) from control levels.

When propranolol (5 mg/kg, ip), the β-adrenergic antagonist, was injected 20 min prior to CC, CC-induced flow from SM and PA glands was markedly reduced; [Ca] was also

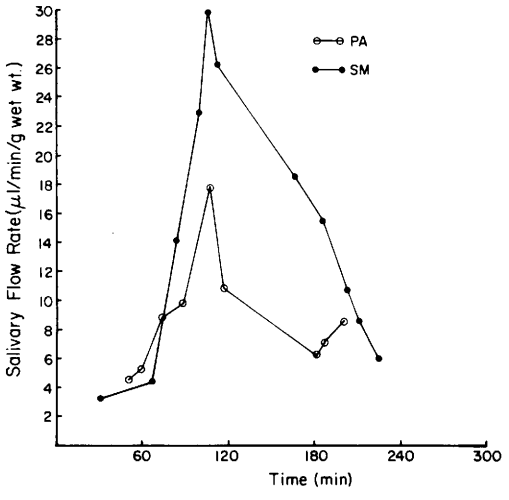

FIG. 1. Time course of change in parotid and submaxillary salivary flow induced by cyclocytidine (CC = 500 mg/kg body wt, ip). Values are representative and are from a single rat.

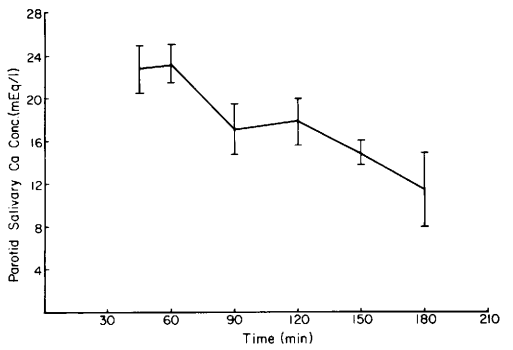

FIG. 2. Time course of change in [Ca] of parotid saliva evoked by CC (dosage as above). Each point represents mean ± SE for at least three rats.

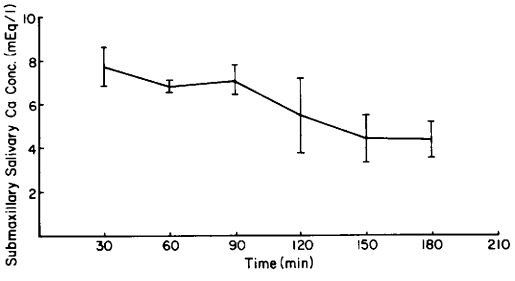

FIG. 3. Time course of change in [Ca] of submaxillary saliva evoked by CC (dosage as above). Each point represents mean ± SE for at least three rats.

reduced from levels seen with CC alone, and, [Ca] of SM saliva was 1.8 meq/liter (two rats each gave this value) as compared with 7 meq/liter with CC alone. Flow from PA was too scanty to provide adequate samples for calcium analysis by methods available in our lab. If propranolol was administered 20 min prior to CC, the expected CC-induced decrease in [Ca] of SM or PA gland did not occur, and values were not different from controls. The acute effects of CC were thus mainly prevented by propranolol.

Because release of NE from sympathetic nerve endings is presumably induced by CC (2, 3), it was postulated that absence of NE would prevent secretion. Therefore reserpine, which causes complete depletion of NE within 24 hr after its administration (10), was given as a single ip dose of 5 mg/kg. No secretion was elicited from either SM or PA of the RES-treated rats when CC was acutely given, nor were glandular levels of calcium reduced. Thus, [Ca] of SM of RES-treated rats was 41.6 ± 1.1 meq/kg wet wt (two rats) while that of SM of RES-treated rats after acute CC stimulation was 38.1 ± 0.9 meq/kg wet wt (two rats).

[Ca] of SM and PA glands was also examined 24 hr after a single injection of CC; [Ca] of SM gland was then more than three times higher than that of controls but that of PA was still reduced slightly. At 48 hr the threefold increase in [Ca] of SM persisted while that of PA was again equal to that of controls (Table I).

When CC was given daily for 3 days, [Ca] of SM gland, measured after 3 days, was again three times higher than that of controls, and PA levels of calcium were equal to levels of controls (Fig. 4). The additional two injections of the drug thus caused no further changes in [Ca], and the maximal increase was evident within 24 hr after a single injection of CC (Fig. 4. and Table I).

To see if the increase in glandular [Ca] induced by CC was related to depletion of NE, RES was given as a single dose 24 hr before CC injection. The data in Table I show that [Ca] of SM gland of CC + RES-treated rats was similar to that of RES-treated rats or CC-treated rats. These values, though equal to each other, were three times as high as those of SM of untreated rats. [Ca] of PA, on the other hand, was very similar with all three drug manipulations (Table I).

Since the effects caused by CC on secretion and growth resemble those produced by β-adrenergic agonists (6, 7), it was postulated that the increase in glandular [Ca] that appeared 24 hr after injection of CC might be mediated by β adrenoceptors. To test this possibility, propranolol was given daily for 3 days 20 min prior to CC. At the end of 3 days, the [Ca] of SM gland of rats treated with propranolol and CC was again three times as high as that of controls but slightly less than values obtained with CC alone (Fig. 4). [Ca] of PA was unchanged from controls. Propranolol thus did not block the CC-induced increase in [Ca] of SM gland. It did, however, block

TABLE I. CALCIUM CONCENTRATION OF SUBMAXILLARY AND PAROTID GLANDS FOLLOWING CC, RES, AND RES + CC

	Parotid gland		Submaxillary gland	
Treatment	Wet wt (mg)	Ca/wet wt (meq/liter)	Wet wt (mg)	Ca/wet wt (meq/liter)
Control	232 ± 7	8.7 ± 0.5 (7)	214 ± 16	11.7 ± 1.3 (6)
CC-24 hr	231 ± 7	8.1 ± 1.4 (3)	191 ± 11	39.4 ± 2.0 (3)
48 hr	297 ± 22*	10.2 ± 1.1 (3)	280 ± 14*	37.8 ± 1.7 (6)
RES-24 hr	175 ± 11	11.6 ± 0.3 (17)	210 ± 6	41.9 ± 0.8 (23)**
48 hr	230 ± 20	13.1 ± 0.7 (3)	251 ± 6	35.0 ± 1.2 (6)**
RES + CC	224 ± 21	11.7 ± 0.3 (3)	235 ± 20	36.3 ± 2.3 (3)**,†

Note. Values are means ± SE. Numbers in parentheses refer to number of rats. CC administered as a single dose of 500 mg, and [Ca] determined at 1 or 2 days later; RES administered as a single dose of 5 mg/kg and [Ca] determined RES + CC (RES first 24 hr, followed by CC next 24 hr). *Statistically significantly different from controls ($P < 0.05$); **$P < 0.001$; †statistical differences between CC, RES, or CC + RES do not exist.

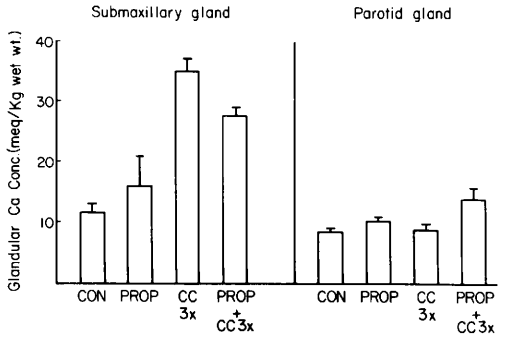

FIG. 4. Effect of propranolol on glandular [Ca] of submaxillary and parotid glands following daily administration (for 3 days) of CC. Con, control; Prop, propranolol (5 mg/kg ip); CC, cyclocytidine (500 mg/kg).

enlargement of both SM and PA glands, and gland weights of rats treated with propranolol and CC were similar to those of controls (Table II).

The increase in wet weight of PA gland after 3 days of CC was much greater than that of SM. Furthermore, propranolol itself appeared to cause a small decrease in weight of both glands; thus, propranolol not only prevented the CC-induced increase in gland weight but even caused a decrease (Table II).

Discussion. Present data confirm previous findings regarding adrenergic actions of CC on SM and PA glands (2, 3), and in addition, show that secretion of calcium, like that of Na and K (2, 3) is the result of adrenergic actions of CC also. [Ca] of saliva evoked by CC is similar to that of saliva elicited by stimulation of the sympathetic nerve (8, 9) and most closely resembles that evoked by isoproterenol stimulation (6, 7). The degree of glandular depletion of calcium following acute stimulation with CC also resembles the depletion caused by stimulation of the sympathetic nerve (nearly 50% within 2 hr) (8, 9). While the effects resemble those induced by stimulation of the sympathetic innervation where NE is released to act on both α and β adrenoceptors, the predominant effect of the NE released by the CC is on β adrenoceptors. Thus, administration of propranolol prior to CC injection largely prevents the adrenergic effects of CC on calcium secretion. From present data it may be postulated that less NE is released by CC than is released normally with stimulation of the sympathetic nerve, and therefore this smaller amount of NE acts almost exclusively on β adrenoceptors (11).

However, the action of CC to cause NE release persists for a long time (6–7 hr) whereas physiological stimulation of the sympathetic nerve causes NE release for a very short period of time (8, 9). Thus, effects of persistent NE stimulation of the glands can be examined using CC. When CC is administered daily over a period of 3 days, the glandular enlargement that follows such treatment can be attributed to the prolonged stimulation of β adrenoceptors by NE. The CC-induced glandular enlargement of PA is much greater than that seen in SM because there are more β adrenoceptors in PA than in SM (12, 13).

A new change induced by CC, that of persistent and large increase in [Ca] of SM gland following chronic administration of the drug, may not be associated with the β-adrenergic action of the drug since propranolol only slightly inhibits the CC-induced increase in glandular [Ca]. The increase in [Ca] of SM gland with CC, as with RES, is probably the result of the same mechanism, i.e., NE depletion. These conclusions are based on the following observations: the magnitude of the [Ca] increase with CC, RES, or RES followed by CC is similar in all three instances; if different mechanisms were involved, the combined effects of RES + CC should be additive.

Present data provide additional evidence that secretory effects of CC are caused by NE released from sympathetic nerve endings (2, 3). Thus secretion is not elicited by CC from

TABLE II. EFFECTS OF CHRONIC ADMINISTRATION OF CYCLOCYTIDINE AND PROPRANOLOL ON WEIGHT OF RAT SALIVARY GLANDS

	Glandular wet wt (mg)	
Treatment	Parotid	Submaxillary
None	232 ± 7 (7)	175 ± 3 (9)
CC-3 days	288 ± 4 (8)**	202 ± 5 (12)**
PROP-3 days	233 ± 27 (3)	165 ± 3 (3)
PROP + CC-3 days	183 ± 14 (5)*	163 ± 5 (4)

Note. Values are means ± SE. Numbers in parentheses refer to number of rats. PROP (5 mg/kg, ip) was given 20 min prior to CC. CC (500 mg/kg) was given daily for 3 days. *Statistically significantly different from controls (no treatment), $P < 0.05$; **$P < 0.001$.

glands of RES-treated rats, since NE levels are depleted by RES (10).

It may be concluded that secretion of calcium as well as secretion of K, Na, and amylase (2) and induction of growth increases (3) are mediated by β-adrenergic effects of the NE released by CC. The increase in glandular calcium of SM is probably not mediated by β adrenoceptors but is a separate phenomenon, possibly related to depletion of NE.

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