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Inhibition of Cyclocytidine-Induced Enlargement of Parotid and Heart by Propranolol or Sympathectomy[1] (39043)

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Cyclocytidine, a potent antitumor agent, has a number of undesirable side actions which include sialorrhea, salivary gland pain, and postural hypotension (1, 2). It was recently shown that the sialorrhea is the consequence of an unexpected β adrenergic effect of the cyclocytidine (3). In this same report, additional side effects of cyclocytidine were described. These included enlargement of salivary glands and heart and occurred after only 3 days of daily administration of the drug. Although this organ hypertrophy was also reminiscent of β adrenergic influences, it was not established that this was indeed a consequence of β adrenergic action. Since organ hypertrophy is a particularly undesirable side action, it was important to determine if it was attributable to the β adrenergic effects, and if so, if it could be prevented by administration of a β antagonist prior to administering of the cyclocytidine. The present work was therefore undertaken to establish these points. It was also the purpose of the present investigation to ascertain how cyclocytidine exerts its β adrenergic effect. It was shown in the previous work that this action is not mediated through central or ganglionic actions (3). It was not, however, established that the action was a direct one at β adrenergic receptors of the effector organs, and an indirect action, involving cyclocytidine-induced release of catecholamines from postganglionic nerve terminals, was suggested. Only preliminary data were available, however, to support this view, and accordingly to elucidate the mode of action of cyclocytidine was also a purpose of the present investigation.

Materials and methods. Female Long-Evans rats 4 months of age and weighing approximately 200 g, and BDF_1 female mice, 2 months old and weighing from 14 to 25 g, were used in these experiments. They were maintained on lab chow and water except for the 18 hr preceding collection of saliva or removal of tissue for enzyme determination, when food but not water, was removed from the animals. Single doses of cyclocytidine, 500 mg/kg, were administered ip for acute experiments; cyclocytidine was also administered chronically (3–5 days) in daily doses of 500 mg/kg, for rats, and 700 mg/kg, for mice. In some rats, propranolol (10 or 20 mg/kg), guanethidine (20 mg/kg), or bretylium (15 mg/kg) was administered 20–30 min prior to injection of the cyclocytidine and this regimen was continued for 3–5 days. In other groups of rats, one superior cervical ganglion was removed, under ether anesthesia, 4–5 days prior to initiation of cyclocytidine injection; the cyclocytidine was administered daily to such rats for 3 days. In the experiments where drug administration continued for 3–5 days, animals were sacrificed 24 hr after the last injection of drugs, and parotid gland and heart ventricles were then removed. They were rapidly weighed on a torsion balance, and then placed in ice-cold 0.4 *N* $HClO_4$ for subsequent extraction of nucleic acids. They were homogenized in 0.4 *N* $HClO_4$, and centrifuged; the supernatant fluid was then discarded. The precipitate was then dispersed, washed three times with cold $HClO_4$, and then hydrolyzed at 90°C for 15 min in 0.4 *N* $HClO_4$. Total nucleic acids were then determined by measuring optical density at 260 nm (4). Total DNA was determined using the diphenylamine reaction (5), and total RNA was determined directly using the orcinol reaction (6) or indirectly by subtracting DNA from total nucleic acids. To test the

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reversibility of the cyclocytidine-induced effects, following a 5-day period of drug injection, rats or mice were maintained, drug free, for an additional 14 days before sacrifice. At this time, organs were removed and treated as already described for determination of weights and nucleic acids.

In acute experiments, designed to observe the secretory effects of cyclocytidine, salivary secretion, stimulated by cyclocytidine, was collected from surgically freed parotid ducts of nembutalized rats and analyzed for amylase activity. Amylase assay was also made on buffered saline homogenates of parotid gland aliquots. Amylase activity, of saliva or tissue, was determined using the method of Myers, Free and Rosinski (7), and expressed as mg reducing substance formed per mg of saliva or gland.

Results. The present data confirm and extend the previous findings concerning effects induced by chronic administration of cyclocytidine. Administration of cyclocytidine, in daily doses of 500 mg/kg, for a period of 3 or 5 days, resulted in enlargement of salivary glands and heart. Within 3 days, weight and total RNA of parotid gland were twice that of glands in untreated rats, and DNA was increased by 19%. The submaxillary gland and heart exhibited less pronounced changes: weight was increased by 25% and 20%, respectively; RNA of heart ventricles was increased 50%, and DNA by 12% ($P < .001$). The changes evident at 3 days were not enhanced by an additional 2 days of treatment and in fact, values for weight and RNA after 5 days were somewhat less than those observed after only 3 days (Table I).

Administration of the β adrenergic antagonist, propranolol, 20–30 min prior to injection of cyclocytidine, completely prevented the expected cyclocytidine-induced enlargement of parotid gland. After 3 or 5 days of such a regimen of drug administration, weight, total DNA and total RNA of rat parotid were not increased above levels exhibited by glands of untreated animals. Furthermore, propranolol caused the same degree of inhibition of the β adrenergic effect of cyclocytidine on parotid, whether administered in 10 or 30 mg/kg dose. Cardiac enlargement was also not induced by chronic treatment with cyclocytidine when propranolol was administered prior to injection of the cyclocytidine. However, complete blockade of the cardiac enlargement was obtained only when the higher doses of propranolol (30 mg/kg) were used. Under these conditions, the values for weight, DNA and RNA were similar to those of untreated rats (Table I). The lower doses of propranolol (10 mg/kg, ip) reduced but did not completely block the β adrenergic effect of cyclocytidine on heart. Propranolol alone did not cause changes in weight, DNA or RNA of

TABLE I. EFFECTS OF CHRONIC ADMINISTRATION OF CYCLOCYTIDINE AND PROPRANOLOL ON WEIGHT AND NUCLEIC ACID CONTENT OF RAT PAROTID AND HEART VENTRICLES.[a]

No. rats	Kind and duration of treatment	Parotid			Heart ventricles		
		Wt (mg)	DNA (μg/gland)	RNA (μg/gland)	Wt (mg)	DNA (μg/heart)	RNA (μg/heart)
6	None	142 ± 2	622 ± 32	1632 ± 80	502 ± 8	673 ± 12	819 ± 27
7	CC-3 days	325 ± 8*	743 ± 42*	3184 ± 53*	590 ± 10*	753 ± 8*	1270 ± 57*
4	IN + CC-3 days$^{\Delta}$	152 ± 19	665 ± 51	1397 ± 107	575 ± 5*	719 ± 19	949 ± 9*
6	CC-5 days	258 ± 23	706 ± 34	2614 ± 80*	557 ± 10*	756 ± 42	940 ± 32*
6	IN + CC-5 days	152 ± 10	699 ± 42	1445 ± 163	483 ± 15	632 ± 31	703 ± 20
6	CC-5 days, None-14 days	186 ± 11*	666 ± 43	2039 ± 180*	622 ± 30*	742 ± 44	1023 ± 36*
4	IN-5 days	135 ± 9	615 ± 40	1384 ± 66	500 ± 15	640 ± 5	819 ± 36
4	IN$^{\Delta}$-5 days	148 ± 4	661 ± 43	1681 ± 111	523 ± 9	635 ± 25	826 ± 31

[a] Values are means ± SE. Dosage of IN (propranolol) = 30 mg/kg except where indicated by Δ where dosage is 10 mg/kg; CC (cyclocytidine), in daily ip doses of 500 mg/kg. Values significantly different from controls (None) ($P < .05$, at least) are indicated by *.

TABLE II. EFFECT OF CHRONIC ADMINISTRATION OF CYCLOCYTIDINE AND PROPRANOLOL ON WEIGHT OF MOUSE PAROTID AND HEART VENTRICLES, AND NUCLEIC ACID CONTENT OF PAROTID.[a]

No. mice	Kind and duration of treatment	Parotid			Body wt (gm)	Heart Vent wt (mg)	Vent wt / Body wt
		Wt (mg)	DNA (μg/gland)	RNA (μg/gland)			
10	None	24 ± 1	152 ± 9	357 ± 17	23 ± 0.6	95 ± 11	4.16 ± .04
6	CC-3 days	42 ± 3*	204 ± 5*	499 ± 32*	15 ± 1.0	76 ± 6	5.08 ± .38*
6	IN + CC-3 days	17 ± 1*	125 ± 5*	227 ± 26*	16 ± 1.2	66 ± .7	4.55 ± .09
6	CC-5 days	47 ± 3	227 ± 5*	607 ± 84*	16 ± 0.4	80 ± 4	5.14 ± .09*
6	IN + CC-5 days	16 ± 2	118 ± 12*	196 ± 29*	16 ± 0.8	76 ± 4	4.85 ± .09*
4	CC-5 days, None-14 days	27 ± 2	170 ± 19	365 ± 36	22 ± 1.0	99 ± 9	4.55 ± .16
3	IN-5 days	20 ± 1	123 ± 2	227 ± 53*	16 ± 0.8	90 ± 2	4.80 ± .08

[a] Values are means ± SE. Dosage of IN (propranolol) = 20 mg/kg; CC (cyclocytidine), in daily ip. doses of 700 mg/kg. Values significantly different from controls (None) ($P < .05$) are indicated by *.

heart or parotid gland, and values with propranolol (at either dose) were not significantly different ($P > .05$) from those of untreated rats.

The permanence of the enlargement caused by administration of cyclocytidine was also examined. After a 5-day period of drug administration, no cyclocytidine was administered for 14 days. Following the drug free period, parotid showed extensive reversal of the cyclocytidine-induced effects, and gland weight was then only 30% higher than that of untreated rats, RNA 25% greater, and DNA levels not significantly different from levels in untreated rats ($P > .05$). With heart, however, the cyclocytidine-induced effects were not readily reversible. Weight, DNA and RNA were similar to ventricular weight, DNA and RNA of rats treated with cyclocytidine for 5 days.

Since mouse is an animal routinely used in studies with antitumor agents, the β adrenergic actions of cyclocytidine were examined in mouse also. The parotid exhibited, as with rat, a doubling of weight after 3 days of administration of cyclocytidine, in daily doses of 700 mg/kg; DNA was increased by 25%, and RNA by 29% (Table II). The values for weight, DNA and RNA after 5 days of treatment were not significantly different from those observed after 3 days of cyclocytidine administration.

When propranolol was administered 20–30 min prior to injection of cyclocytidine, the expected increases in gland weight, DNA and RNA induced by cyclocytidine did not occur. Values for weight, DNA and RNA were equal to or even somewhat less than those of untreated animals (Table II). Gland weight, DNA and RNA of mice treated only with propranolol were not different from values for weight, DNA and RNA of mice treated with propranolol plus cyclocytidine, but were lower than those of untreated mice (Table II). The propranolol prevented the β adrenergic action of cyclocytidine and possibly exerted some slight additional inhibitory effect.

The chronic effects of cyclocytidine on mouse parotid were almost completely reversed 14 days after cessation of cyclocytidine administration (Table II). In fact, values for gland weight, DNA and RNA were not significantly ($P > .05$) different from those of untreated mice.

The drug regimen itself caused little change in body weight of rats (3), but it caused a significant reduction in body weight of mice (30–35%). Thus, since the effects of cyclocytidine on heart were, even in rat, modest when compared with the effects on salivary glands, this decrease in body weight had to be considered in assessing the influence of cyclocytidine on mouse heart. Thus, even though the absolute weights of mouse ventricles after 3 days of administration of cyclocytidine were less than those of untreated mice, when the change in body weight was con-

sidered, and ventricular weight related to body weight, cyclocytidine caused an increase in heart weight. The ratio of ventricular to body weight for the cyclocytidine treated mice was 5.08, whereas for untreated mice it was 4.12 (Table II). The same magnitude of increase (18%) was observed after 5 days of cyclocytidine tratment also (Table II).

The β adrenergic effects of cyclocytidine are not indirectly mediated through central or ganglionic actions (3). It remains possible, however, that cyclocytidine causes β adrenergic effects by some other indirect action, such as inducing release of catecholamines from postganglionic nerve fibers. From preliminary data previously reported such a mode of action appeared probable (3). These data showed that 2 weeks after removal of the superior cervical ganglion when degeneration of the postganglionic nerve terminals had occurred (8) some saliva was evoked from the sympathectomized as well as the innervated parotid by cyclocytidine. However, the secretion from both glands in each of two rats was scanty; furthermore, no analyses were made that would reflect the magnitude or kind of secretory response elicited. In addition, these experiments were performed after 2 weeks of denervation when atrophic changes (9) which further complicate interpretation of functional changes, had already occurred. Therefore, it was the objective of the present experiments to determine whether or not cyclocytidine causes its β adrenergic effects by an indirect action involving release of catecholamines from postganglionic nerve fibers. Accordingly, the superior cervical ganglion was removed and 4–5 days later, cyclocytidine was administered acutely or over a period of 3 days. At this time following sympathectomy, catecholamine depletion in nerve terminals has occurred (8), but atrophy is not evident; the secretory characteristics were assayed following cyclocytidine administration to determine the kind of receptors stimulated. Under these circumstances, cyclocytidine evoked a scanty flow of saliva from both denervated and innervated glands, but quantities from the denervated glands were too small for analysis. Cyclocytidine-evoked saliva from the innervated glands showed the usual high levels of amylase (700–800 mg/mg saliva) (3). Two hours after administration of cyclocytidine, parotid glands were analyzed for amylase activity. There was the expected marked depletion of amylase in the innervated parotid (3), but the levels in the denervated gland were very little changed from the levels in the unstimulated gland (Table III). These results suggested that cyclocytidine exerted its adrenergic effects indirectly, possibly by causing release of catecholamines (principally norepinephrine) from nerve terminals, since in the sympathectomized glands where catecholamines were depleted, cyclocytidine did not have adrenergic effects (10).

As a further test of this hypothesis, cyclocytidine was administered for 3 days and salivary glands and heart were then examined for evidence of enlargement. The data in Table IV show that the cyclocytidine caused the expected 100% increases in weight and RNA, and the expected 19% increase in DNA in the innervated parotid gland but the sympathectomized parotid exhibited only small increases in weight, DNA and RNA (21–12%, respectively). The cyclocytidine-induced increases in weight, DNA and RNA of the heart ventricles were, as expected virtually identical to those induced by cyclocytidine in unoperated rats.

Finally two drugs whose mode of action is at least in part, to prevent release of norepinephrine from nerve fibers (8), were used in combination with cyclocytidine. Guanethidine (20 mg/kg) or bretylium (15 mg/kg) was injected in rats 25-min prior to daily administration of 100 mg of cyclocytidine. After 3 days of the combined drug regimen, weight and nucleic acid content of parotid glands and heart ventricles were determined. It is clear from the data in Table V that cylocytidine did not cause the usual degree of heart enlargement when either guanethidine or bretylium was administered in conjunction with it; a partial blockade of the cyclocytidine effects was also observed in parotid, but only with guanethidine and not with bretylium (Table V).

Discussion. Parotid gland and heart of mouse as well as rat exhibit enlargement

TABLE III. EFFECTS OF ACUTE STIMULATION WITH CYCLOCYTIDINE ON AMYLASE ACTIVITY OF SYMPATHECTOMIZED PAROTID OF RAT[a].

	Amylase Activity of parotid gland (mg/mg)	
	Unstim	Cyclocytidine stim (2 hr)
Sx	590 ± 58 (3)	517 ± 47 (3)
Inn	565 ± 34	190 ± 57
Un Inn	525 ± 30 (3)	80 ± 23 (4)

[a] Values are means ± SE. Sx = left superior cervical ganglion removed 5 days prior to gland removal; Inn = innervated mate; Un Inn = innervated glands of unoperated rats. No. in parentheses refers to number of rats. 100 mg cyclocytidine injected 2 hr prior to gland removal. Amylase activity expressed as mg reducing substance formed per mg wet tissue.

TABLE IV. EFFECTS OF CHRONIC ADMINISTRATION OF CYCLOCYTIDINE ON WEIGHT AND NUCLEIC ACID CONTENT OF SYMPATHECTOMIZED PAROTID OF RAT.[a]

Kind and duration of treatment	Parotid		
	Wt (mg)	DNA (μg/gland)	RNA (μg/gland)
Sx, CC-3 days	180 ± 13	660 ± 31	1812 ± 74
Inn, CC-3 days	310 ± 12	738 ± 24	3254 ± 161

[a] Values are means ± SE from 10 rats. Left superior cervical ganglion removed 5 days prior to initiation of cyclocytidine (CC) regimen (daily ip. injections of 500 mg/kg for 3 days); denervated parotid indicated, Sx; innervated mate, Inn.

following chronic administration of cyclocytidine. The increases in these organs induced by chronic administration of cyclocytidine appear to be, in both species, the result of a β adrenergic action of this agent since these effects were prevented when the β adrenergic antagonist propranolol was administrered just before the injection of the cyclocytidine. Since the β adrenergic property of the cyclocytidine is separate from its antitumor property (11), this undesirable β adrenergically induced enlargement of salivary glands and heart as well as other undesirable β adrenergically induced side actions (1–3) associated with cyclocytidine administration can be eliminated or at least controlled by judicious use of propranolol with cyclocytidine without compromising the carcinolytic action of the cyclocytidine.

The enlargement caused by cyclocytidine appears to reach a maximum very soon after initiation of drug administration (3 days). However, while this suggests that continued use of the cyclocytidine therapeutically should not be expected to cause progressive increase in organ size, the increases that do occur, especially with heart, are not rapidly reversed upon cessation of drug administration. For effective control judicious use of propranolol with the cyclocytidine is thus again indicated.

The present work also indicates that cyclocytidine induces much of its β adrenergic effects by an indirect action, and that this indirect action probably involves cyclocytidine-induced release of catecholamines from postganglionic nerve fibers. This conclusion is supported by the finding that, while secretory effects elicited by cyclocytidine are identical in acutely sympathectomized and innervated parotid (3), the elicited secretory effects in the chronically (5 days) sympathectomized and innervated glands differ markedly from each other. With acute sympathectomy, norepinephrine of postganglionic nerve fibers is unaltered two hours after removal of the superior cervical ganglion and therefore cyclocytidine can induce the same release of catecholamine from the cut as it does from intact postganglionic nerve fibers; however, 5 days after sympathectomy, norepinephrine levels of the fibers are depleted (8), and accordingly, since norepinephrine is, under such conditions not available for release, no β adrenergic effects are induced by cyclocytidine action. It should be pointed out that adrenergic neurotransmitter released from nerve terminals by cyclocytidine acts on α as well as β adrenergic receptors of the gland, but that the β effects predominate (3).

The effects of cyclocytidine on gland size are also altered in the chronically sympathectomized parotid and this finding provides further evidence that cyclocytidine causes β adrenergic effects by an indirect action involving release of catecholamines from post-

TABLE V. MODIFICATION OF EFFECTS OF CHRONIC ADMINISTRATION OF CYCLOCYTIDINE ON WEIGHT AND NUCLEIC ACID CONTENT BY PRIOR INJECTION OF GUANETHIDINE OR BRETYLIUM.[a]

No. rats	Kind and duration of treatment	Parotid			Heart ventricles		
		Wt (mg)	DNA (μg/gland)	RNA (μg/gland)	Wt (mg)	DNA (μg/gland)	RNA (μg/gland)
2	CC-3 days	372 ± 4	773 ± 53	4417 ± 169	655 ± 25	803 ± 4	1518 ± 70
3	Guan. + CC-3 days	281 ± 27*	702 ± 62	3366 ± 285*	550 ± 15*	660 ± 10*	1058 ± 21*
2	Bret. + CC-3 days	402 ± 24	845 ± 90	4606 ± 495	580 ± 10	683 ± 18*	1220 ± 3*

[a] Values are means ± SE. Guanethidine (Guan, 20 mg/kg), or bretylium (Bret, 15 mg/kg) administered daily ip. 20′–30′ prior to injection of cyclocytidine (CC, 500 mg/kg), for 3 days. Values significantly different from cyclocytidine group indicated with *.

ganglionic nerve fibers. Thus, in the chronically sympathectomized parotid where norepinephrine of the postganglionic fibers and gland is depleted (8), cyclocytidine did not induce the glandular enlargement attributed to its β adrenergic action. Again, norepinephrine was not available for release and subsequent action at β receptors of the gland.

Since gland levels of catecholamines are also affected by chronic sympathectomy, these data, while suggestive, did not establish conclusively that the cyclocytidine causes β adrenergic effects on parotid by inducing release of norepinephrine from the nerve endings, nor did they show that cyclocytidine induced release of norepinephrine from postganglionic fibers was involved in the β adrenergically induced heart enlargement. Therefore, more definitive delineation of the mode of action was sought by use of agents that block release of norepinephrine from postganglionic nerve fibers (8, 12). The cyclotidine-induced enlargement of heart could be partially blocked when drugs that prevent release of norepinephrine from postganglionic nerve fibers are used with the cyclocytidine. This partial blockade was obtained with both guanethidine and bretylium but the parotid enlargement was blocked (and then only partially) with guanethidine only. The present findings with guanethidine and bretylium must be somewhat cautiously interpreted since the actions of these agents include, in addition to causing a decreased release of adrenergic mediator (8, 12), direct and indirect sympathomimetic actions, and a supersensitivity of effector cells that is similar to that caused by sympathetic postganglionic denervation (13). While the present work does not conclusively establish the mechanism by which cyclocytidine exerts its β adrenergic effects, these actions are at least to a great extent mediated indirectly, and the evidence supports the view that cyclocytidine does this by causing release of adrenergic mediators from postganglionic nerve terminals.

Summary. Enlargement of salivary glands and heart of mouse as well as rat is caused by chronic administration of the antitumor agent cyclocytidine. In rat, the effects are maximal within 3 days, and are reversible, but with heart, not readily so. The organ enlargement, in both species, is the result of an action of the cyclocytidine involving β adrenergic receptors since administration of propranolol 20 min prior to injection of cyclocytidine prevented the enlargement as well as the increases in nucleic acids associated with the enlargement. These β adrenergic effects appear mediated through indirect actions of cyclocytidine that probably involve release of norepinephrine from postganglionic nerve fibers. This conclusion is based on the findings that parotid sympathectomized for 5 days does not exhibit the same secretory responses and glandular enlargement with cyclocytidine administration that are observed in the innervated mate. For example, amylase activity of the innervated gland was reduced from 525 mg/mg of gland (unstimu-

lated parotid) to approximately 190 two hr after administration of 500 mg/kg of cyclocytidine, whereas that of the sympathectomized gland was unchanged (510 mg/mg). In addition, cyclocytidine caused only a small increase in weight and nucleic acid content of the sympathectomized parotid, whereas the innervated mate exhibited the usual marked increases in all parameters (weight and RNA were twice as great as those of untreated rats). Guanethidine and bretylium were used in conjunction with cyclocytidine to provide additional evidence for the indirect β adrenergic action of cyclocytidine on heart and parotid gland.

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