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Mechanism of the Cardiovascular Actions of Cyclocytidine (40351)

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0^2,2′-cyclocytidine was synthesized to provide a useful depot form of the antineoplastic agent, arabinofuranosylcytosine (ara-C). Although one of the primary antileukemic drugs currently available (1), ara-C must be administered by frequent intermittent or continuous intravenous infusion to maintain effective plasma levels because it is rapidly inactivated by deamination in the body (2). Cyclocytidine, an anhydride analogue of ara-C, is hydrolyzed to ara-C *in vivo* and requires only once daily intravenous administration to maintain adequate ara-C plasma levels (3). In doses of 300–600 mg/m^2, cyclocytidine has shown promise in the treatment of acute myelogenous leukemia in man (4). Unfortunately, cyclocytidine produces unusual side effects which limit its clinical use (5). The most pronounced undesirable side effects are sialorrhea, parotid pain and, especially, acute cardiovascular effects characterized by postural hypotension leading to syncope. Although cyclocytidine is considered overall to afford a more favorable therapeutic index than ara-C, the postural hypotension and other side effects produce sufficient patient discomfort to hamper its acceptability. The present investigation was initiated to determine the mechanism by which cyclocytidine affects function of the mammalian cardiovascular system.

Materials and methods. Experiments were conducted with anesthetized dogs, cats and rats. Beagle dogs of either sex (supplied by the Laboratory of Toxicology, National Cancer Institute), weighing 9–11 kg, were anesthetized with barbital sodium (250 mg/kg) and thiopental sodium (15 mg/kg) administered intravenously. Cats of either sex, weighing 2.3–3.5 kg, were anesthetized with the barbital–thiopental mixture given either intravenously or intraperitoneally. Male Sprague-Dawley rats, weighing 150–200 g, were anesthetized with pentobarbital sodium (45 mg/kg) given intraperitoneally. Animals were allowed to breathe spontaneously through endotracheal tubes (dogs and cats) or through polyethylene tracheal cannulae (rats). Femoral arteries (dogs and cats) or carotid arteries (dogs and rats) were cannulated with heparin–saline filled polyethylene catheters. Systemic arterial blood pressure was measured by a Statham P23Db pressure transducer connected to a Beckman type RM oscillographic recorder. Drugs, dissolved in 0.9% sodium chloride solution, were administered into a cannulated femoral vein (dogs and cats) or jugular vein (rats) in volumes of 0.1–1 ml/kg. Blood pressure responses were measured as maximum changes in systolic pressure.

Postural hypotension was evaluated by tolerance of dogs to head-up tilt. In the tilt studies, blood pressure was measured from carotid arteries. Dogs were fastened securely to a conventional metal surgical board and one end of the board was elevated to a predetermined height for 60 sec; the angle of tilt was 20° from horizontal. The time required for restoration of systolic blood pressure to one-half of the change from pretilt values was taken as the index of tolerance to tilt.

Drugs used were cyclocytidine HCl (Drug Development Branch, Division of Cancer Treatment, National Cancer Institute), l-norepinephrine HCl (Levophed, Winthrop), tyramine HCl (Aldrich), hexamethonium chloride (City Chemical Corp.), phentolamine HCl (Regitine, Ciba), propranolol HCl (Inderal, Ayerst), guanethidine HCl (Ismelin, Ciba), desmethylimipramine HCl (Desipramine, Geigy Pharmaceuticals), and 6-hydroxydopamine HBr (Regis). All dosages were calculated as the salt forms. Statistical analyses were performed by use of the Student's *t* test, group comparisons or paired comparisons; values of P equal to or less than 0.05 were considered significant.

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Results. Cyclocytidine, in bolus doses of 5–100 mg/kg, increased blood pressure in dogs, cats and rats (Fig. 1). The pressor responses, which consisted of increases in both systolic and diastolic pressures, were transient and, depending on the dose of cyclocytidine, generally returned to preinjection values within 5–15 min. Responses to the highest doses of cyclocytidine often persisted for 30–60 min. The magnitudes of the pressor responses depended both on dosage and order of administration. Responses to initial injections were dose-related in all three species up to 100 mg/kg, the largest dose tested. Repeated injections in the same animal, however, revealed varying degrees of tachyphylaxis to the pressor effects of cyclocytidine. In dogs, separate injections of 5 mg/kg of cyclocytidine produced equivalent increases in blood pressure (Fig. 2). After a cumulative dosage of 80 mg/kg, bolus injections of 80 mg/kg raised blood pressure, but the magnitude of the increase was reduced in comparison to a previous injection of 60 mg/kg. In rats, second doses of 25 mg/kg caused less increase in blood pressure than initial doses of 5 mg/kg (Fig. 2). After a cumulative dosage of 30 or 130 mg/kg, bolus injections of 100 mg/kg of cyclocytidine elicited less pressor effect than initial doses of 5 mg/kg.

Six dogs were tested for tolerance to tilt before and after treatment with cumulative doses of 60 mg/kg of cyclocytidine. Before treatment, the dogs regained 50% of the pretilt carotid blood pressure within 17 ± 9 sec. After treatment with cyclocytidine, the time required for 50% recovery of carotid systolic pressure was 41 ± 9 sec.

Experiments with a ganglionic blocker and antiadrenergic drugs were conducted to localize anatomically the site of the pressor action of cyclocytidine and to determine the mechanism by which this vasopressor drug causes postural hypotension. Administration of the ganglion blocking drug, hexamethonium (20 mg/kg), did not reduce subsequent (10–20 min) pressor responses to cyclocytidine in dogs (Fig. 3), cats or rats. Responses to cyclocytidine were, however, antagonized slightly by prior administration of propranolol (0.5 mg/kg) and were essentially abolished by the alpha adrenergic receptor antagonist, phentolamine (2 mg/kg) (Fig. 3). These results suggested that cyclocytidine causes pressor responses either directly by acting upon cardiac and vascular adrenergic receptors or indirectly by promoting release of endogenous adrenergic amines. Acute administration (10–30 min before cyclocytidine) of guanethidine (2 mg/kg) completely obliterated pressor responses to cyclocytidine (Fig. 3). Desmethylimipramine (10 mg/kg) also blocked pressor responses to cyclocytidine in dogs and in rats (Fig. 3 and Table I). To establish conclusively that pressor responses to cyclocytidine result from release of norepinephrine from adrenergic neurons, rats were injected with 6-hydroxydopamine (100 mg/kg) 24 hr in advance to disrupt function of adrenergic fibers. Pressor responses to cyclocytidine and to tyramine were compared in control and in 6-hydroxydopamine-treated animals. Prior treatment with 6-hydroxydopamine nearly abolished pressor responses to tyramine and to cyclocytidine (Table II).

Pressor responses to norepinephrine and to tyramine were measured before and after acute administration of 100 mg/kg of cyclocytidine. Responses to norepinephrine were not altered by cyclocytidine treatment, but responses to tyramine were significantly reduced (Fig. 4). Pressor responses to tyramine and to cyclocytidine were not altered in animals injected 24 or 48 hr previously with 100 mg/kg of cyclocytidine.

Discussion. In humans, cyclocytidine causes profound changes in cardiovascular function in the usual therapeutic dose of 8–16 mg/kg (5). In this same general range of dosage, cyclocytidine causes increases in systemic blood pressure in dogs, cats and rats and, in dogs, induces cardiovascular intolerance to head-up tilt. Possible sites of cardiovascular action of cyclocytidine included baroreceptor and chemoreceptor reflex mechanisms, the central nervous system, sympathetic ganglia, adrenergic nerve terminals, adrenergic receptors and vascular smooth muscle.

The failure of hexamethonium to alter pressor responses to cyclocytidine eliminated the baroreceptor and chemoreceptor reflexes, the central nervous system, and sympathetic ganglia as potential sites of action. The pressor effects of cyclocytidine were blocked by phentolamine, indicating that it acts directly

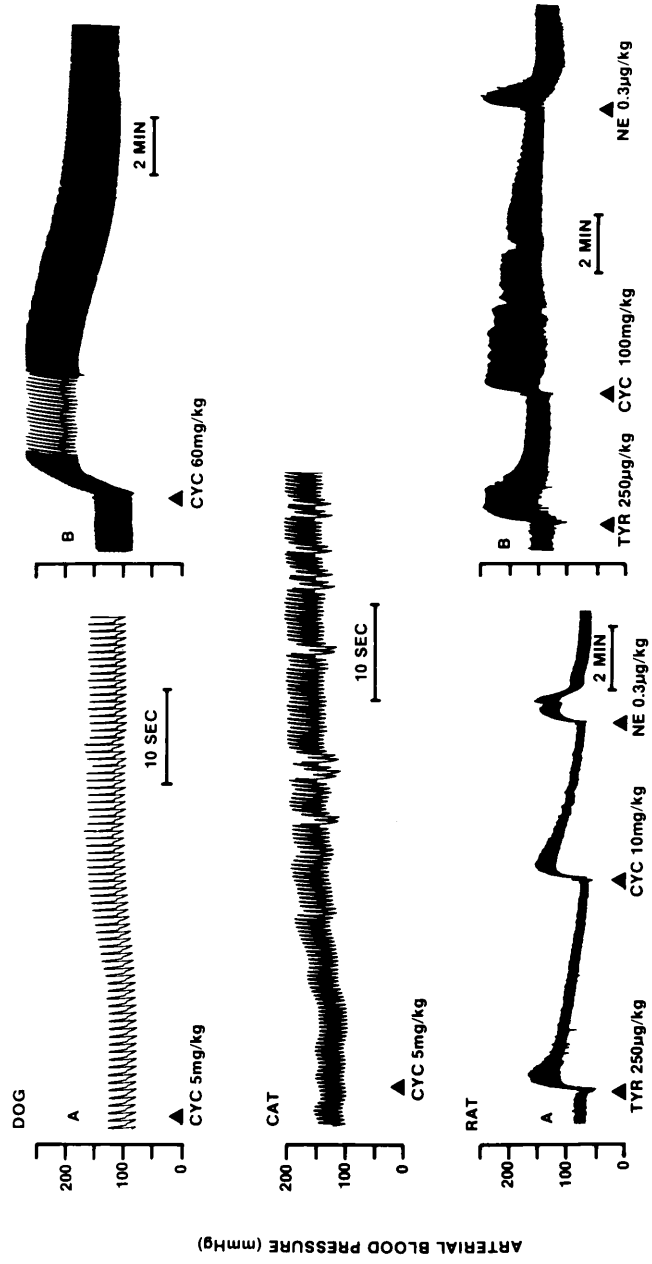

FIG. 1. Blood pressure responses to cyclocytidine (CYC) in three species. DOG. As can be seen in panel A, cyclocytidine had little effect on heart rate in dogs. Panel B shows the response to a larger initial dosage of cyclocytidine in the dog; the chart speed was increased briefly to allow counting of heart rate. CAT. The blood pressure record from the cat shows reflex slowing of the heart during the height of the pressor response. RAT. In the rat, pressor responses to 10–100 mg/kg of cyclocytidine are equivalent to those induced by 250 µg/kg of tyramine (TYR) or 0.3 µg/kg of norepinephrine (NE). In the record in panel A, all pressor agents increased both diastolic and systolic pressure. In panel B, the three agents increased systolic pressure more than diastolic pressure. Blood pressure was recorded from femoral arteries in dogs and cats, from carotid arteries in rats.

or indirectly upon vascular alpha adrenergic receptors and not upon nonadrenergic vascular elements. The rapid tachyphylaxis to its pressor effects suggested that cyclocytidine could act indirectly by promoting release of norepinephrine from labile neuronal sites. Blockade of the pressor effects of cyclocytidine by guanethidine, which interferes with the adrenergic nerve uptake system and has norepinephrine antirelease properties, confirmed the adrenergic nerve as the site of cyclocytidine pressor effects. Blockade of cyclocytidine pressor effects by desmethylimipramine, which has little antirelease activity, could be explained by prevention of cyclocytidine entry into the adrenergic neurons (6). Finally, depletion of neuronal norepinephrine by 6-hydroxydopamine (7) demonstrated that once cyclocytidine enters adrenergic nerves, it acts by release of endogenous norepinephrine. Similar mechanisms may explain the actions of cyclocytidine on rat salivary glands, where salivation is blocked by propranolol, but not by acute sympathetic ganglionectomy (8, 9).

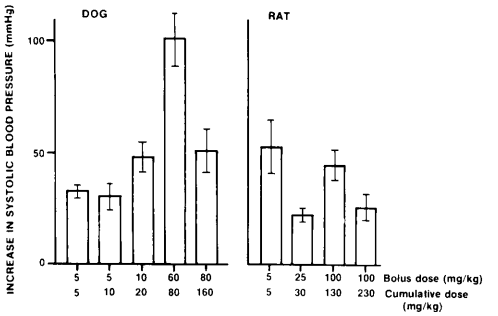

FIG. 2. Pressor responses to repeated doses of cyclocytidine in dogs ($N = 6$) and rats ($N = 6$). Each bar is the mean ± SEM of the increases in systolic blood pressure.

Cyclocytidine reduced cardiovascular tolerance to tilt, the correlate in dogs of postural hypotension in humans. The postural hypotension induced by cyclocytidine does not result from blockade of adrenergic receptors, but rather from interference with adrenergic neurons. This was shown by loss of responsiveness to tyramine, but not to norepinephrine, after acute administration of cyclocytidine. The effects of cyclocytidine on adrener-

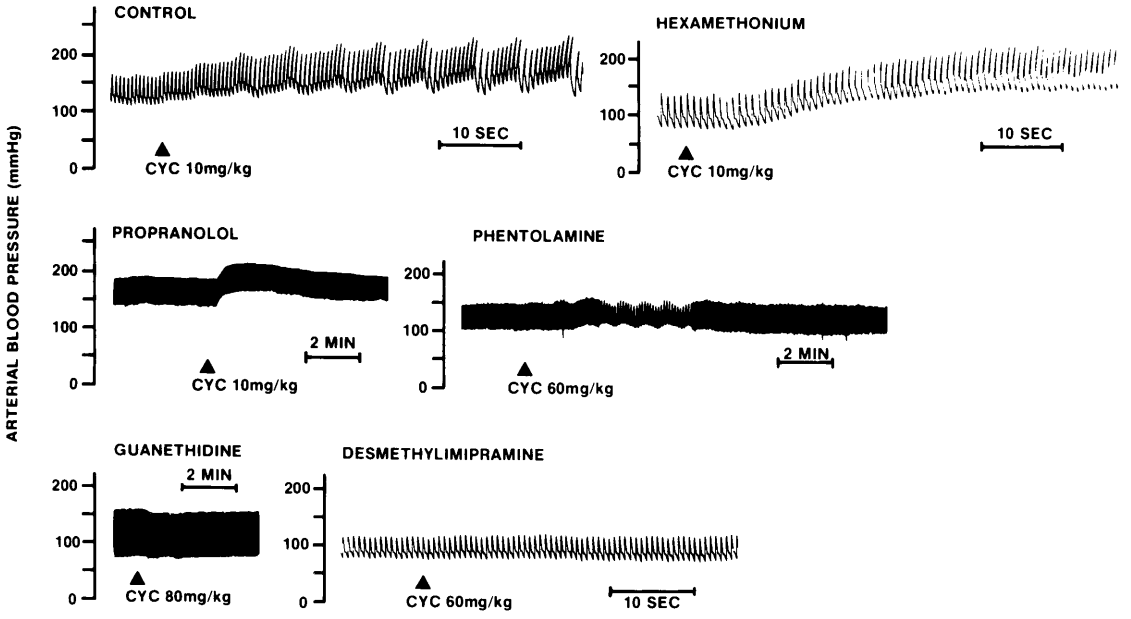

FIG. 3. Blood pressure responses to cyclocytidine (CYC) in dogs under control conditions and 10–20 min after administration of hexamethonium (20 mg/kg), propranolol (0.5 mg/kg), phentolamine (2 mg/kg), guanethidine (2 mg/kg) or desmethylimipramine (10 mg/kg). Reflex cardiac slowing is evident during the height of the control pressor response to cyclocytidine, but not in the animal treated with hexamethonium. Treatment with phentolamine, guanethidine or desmethylimipramine virtually abolished pressor effects of cyclocytidine. Blood pressure was recorded from femoral arteries.

TABLE I. EFFECTS OF DESMETHYLIMIPRAMINE (DMI) ON PRESSOR RESPONSES TO CYCLOCYTIDINE.

Species	Dose of cyclocytidine	Control animals N	Control animals Increase in b.p. (mm Hg)[a]	Animals treated with DMI (10 mg/kg) N	Animals treated with DMI (10 mg/kg) Increase in b.p. (mm Hg)[a]	P
Dog	60 mg/kg	5	101 ± 12	5	6 ± 4	<0.01
Rat	100 mg/kg	6	45 ± 7	5	16 ± 6	<0.01

[a] Mean ± SEM.

TABLE II. EFFECTS OF 6-HYDROXYDOPAMINE (6-OHDA) ON PRESSOR RESPONSES TO TYRAMINE AND CYCLOCYTIDINE IN RATS.

	Control animals N	Control animals Increase in b.p. (mm Hg)[b]	Animals after 6-OHDA[a] N	Animals after 6-OHDA[a] Increase in b.p. (mm Hg)[b]	P
Tyramine 200 μg/kg	6	75 ± 11	3	6 ± 3	<0.05
Cyclocytidine 5 mg/kg	6	53 ± 12	3	2 ± 2	<0.05

[a] 6-OHDA (100 mg/kg) administered 24 hr before experiment.
[b] Mean ± SEM.

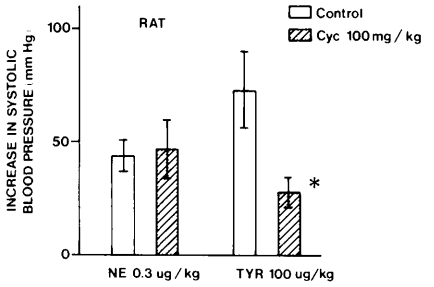

FIG. 4. Pressor responses in rats to norepinephrine (NE) and to tyramine (TYR) before (Control) and 10–30 min after administration of cyclocytidine (CYC). *, decreased significantly from control.

gic vasoconstrictor neurons are temporary and disappear within 24 hr.

Based on these observations, we propose that acutely administered cyclocytidine enters adrenergic nerve terminals and initially promotes release of norepinephrine from a labile functional pool (10). This action causes a transient, dose-related increase in blood pressure. After the most labile pool of norepinephrine has been mobilized, the intraneuronal cyclocytidine inhibits temporarily further secretion of norepinephrine from the nerves. Responses to subsequent doses of cyclocytidine (or tyramine) are thereby inhibited. Postural hypotension could be explained by cyclocytidine-induced temporary failure of the adrenergic neuronal elements which participate in the reflex adjustments of the cardiovascular system required for maintenance of blood pressure in response to gravitational stress.

Summary. The clinical usefulness of cyclocytidine, an otherwise potentially valuable antineoplastic agent, is limited because it may cause acute postural hypotension in man. In the laboratory, cyclocytidine (5–100 mg/kg) transiently increased blood pressure in anesthetized dogs, cats and rats. As the pressor responses to cyclocytidine were prevented by previous treatment with 6-hydroxydopamine or acutely by phentolamine, guanethidine and desmethylimipramine, but not by hexamethonium, adrenergic nerve terminals appear to be involved in its pressor actions. Cyclocytidine also blocked pressor responses to tyramine and caused intolerance to tilt stress in anesthetized dogs. Cyclocytidine thus appears to promote, then prevent, release of norepinephrine from adrenergic vasoconstrictor neurons.

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