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Cardiovascular Actions and Interactions of Chlordimeform in the Dog

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Chlordimeform, N'-(4-chloro-o-tolyl)-N, N-dimethylformamidine, CDF, Galecron^R, Fundal^R, is a member of a relatively new class of acaricidal/pesticidal compounds referred to as formamidines. CDF possesses several pharmacological actions that implicate the cardiovascular system as a potential target organ in acute poisoning. MATSUMURA and BEEMAN (1976) reported a profound fall in blood pressure in rabbits following administration of a large (200 mg/kg i.p.), single dose of CDF. It has also been reported (LUND et al. 1978) that CDF depresses cardiac contractility and reduces hindlimb perfusion pressure in anesthetized dogs. Contractions of isolated rabbit aortic strips elicited by potassium, histamine, serotonin and norepinephrine were antagonized by CDF (ZELENSKI et al. 1978). ROBINSON and BITTLE (1979) reported demethylchlordimeform, a metabolite of CDF, caused contraction of rabbit central ear artery strips in concentrations of 10^{-9} to 10^{-4} M. CDF has been shown to inhibit MAO and alter brain amine levels by numerous investigators (AZIZ and KNOWLES 1973, BEEMAN and MATSUMURA 1973, BENEZET and KNOWLES 1976, MAITRE et al. 1978, RIEGER et al. 1980). Several authors have concluded it is not the primary cause of death in acute intoxication with formamidines, (ROBINSON et al. 1975, ROBINSON and SMITH 1977, HOLLINGSWORTH et al. 1979) although this would not preclude an alteration of cardiovascular function. The purpose of our investigation was to acquire additional quantitative information of CDF's effects on the mammalian cardiovascular system and to ascertain whether CDF acts directly or through an amine mediated mechanism.

METHODS

Mongrel dogs of either sex weighing between 14 and 23 kg were used in this study. All were vaccinated against canine distemper and infectious canine hepatitis, were free from internal and external parasites, and had normal hematocrit values. Dogs were anesthetized with 30 mg/kg pentobarbital sodium administered i.v. and were given supplements as needed throughout the experiment. The right cephalic vein and left femoral artery were cannulated for drug injections and blood pressure recording, respectively. The right femoral artery was exposed and a blood flow transducer placed around it. Musculocutaneous blood flow to the right hind limb, systolic and diastolic blood pressure, and heart rate were continously recorded. Heparin sodium was routinely administered to prevent clotting of blood.

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In the first set of experiments, dogs were given a total of eleven increasingly larger doses of CDF at approximately 3 min intervals, starting with 0.01 mg/kg and doubling each successive dose until a final dose of 10.2 mg/kg had been given. In some instances the beta-adrenergic blocking agent propranolol (0.5 mg/kg) and the muscarinic blocking agent atropine (2.0 mg/kg) were given prior to CDF administration. Cardiovascular changes elicited by giving the series of eleven increasingly larger doses of CDF in the presence of these blockers were compared with those obtained in dogs not given the blocking agents. Single doses of CDF (20.5 and 30.0 mg/kg) were given to dogs in another set of experiments.

Attempts were made to determine whether CDF would modify changes in blood pressure, heart rate, or blood flow elicited by i.v. administration of several cardio-vasoactive agents. Five drugs, l-norepinephrine HCl (1 ug/kg), isoproterenol HCl (1 ug/kg), acetylcholine chloride (0.5 ug/kg), l-epinephrine bitartrate (2 ug/kg), and histamine dihydrochloride (10 ug/kg), were used. Control responses to these five agents were first obtained, then a single dose of CDF was administered (20.5 mg/kg i.v.), and after a 10 to 15 minute stabilization period administration of the five agents was repeated.

Blood flow rates were determined with a Narcomatic Electromagnetic Flowmeter, Model RT 500 (Narco-biosystems, Houston, TX) and appropriate flow transducers. Blood pressure and heart rate were recorded with a pressure transducer (pulsatile setting), Model P-1000 A (Narco-biosystems). Changes in systolic and diastolic pressure, heart rate, and blood flow were recorded simultaneously and continuously on a DMP-4B Physiograph (Narco-biosystems). Drug solutions for injection were prepared in saline just prior to use. Injection volumes were kept below 1 ml, except when CDF was administered in doses of 5 mg/kg or larger. Each injection was followed immediately by a 1.5 ml saline flush to empty the cannula.

Peak responses, regardless of time of occurrence, were used in computing results. Comparisons of means for significance of differences were made with the Student's "t" test for non-paired data when analyzing the cardiovascular changes elicited by CDF, alone and in the presence of propranolol and atropine. The Student's "t" test for paired data was utilized to determine whether CDF enhanced or diminished the cardiovascular effects of the five cardio-vasoactive agents. $P \leq .05$ was considered significant.

RESULTS

Typical CDF induced changes in cardiovascular parameters are shown in Fig. 1. Peak responses were observed within seconds, 26 ± 2.1 for changes in pressure (systolic and diastolic), 27 ± 2.0 for the initial decrease in flow, and 42 ± 1.9 for the secondary increase in flow, for data obtained in the initial series of experiments (eleven increasingly larger doses of CDF). Responses were dose dependent and relatively brief in duration, when considering all doses of CDF administered.

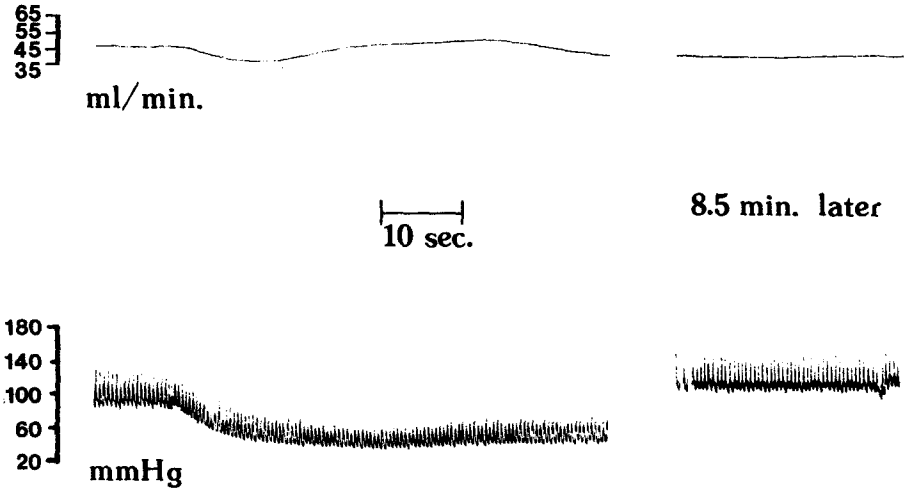

Fig. 1. Effect of 10.2 mg/kg CDF administered 10 sec. before start of tracing on blood flow (upper) and blood pressure (lower) recorded from femoral artery of anesthetized dog.

TABLE I

Cardiovascular changes induced by i.v. administration of CDF to anesthetized dogs. Values represent mean percent change in peak response.

	Dose of CDF		
Parameter Measured	10.2 mg/kg[1] (N=5)	20.5 mg/kg[2] (N=8)	30.5 mg/kg[2] (N=3)
Systolic Blood Pressure	28	34	57
Diastolic Blood Pressure	26	44	68
Heart Rate	6	12	0.6
Blood Flow, Initial Change	36	62	
Blood Flow, Secondary Change	31	127	

(1) Last dose of a series of eleven increasingly larger doses.
(2) Single bolus dose.

Reductions in diastolic and systolic pressures were observed when as little as 5.1 mg/kg of CDF was administered (Fig. 2A, 2B). Peak reductions of 28 and 26 percent in systolic and diastolic pressures, respectively, were seen when the last dose (10.2 mg/kg) of the series of eleven injections was given. The magnitude of the depressor response was not affected by the presence of atropine or propranolol (Fig. 2A, 2B). Reductions of 34 and 44 percent in systolic and diastolic pressures were observed in dogs given single doses of 20.5 mg/kg CDF, and 57 and 68 percent, respectively, when given 30.0 mg/kg (Table I).

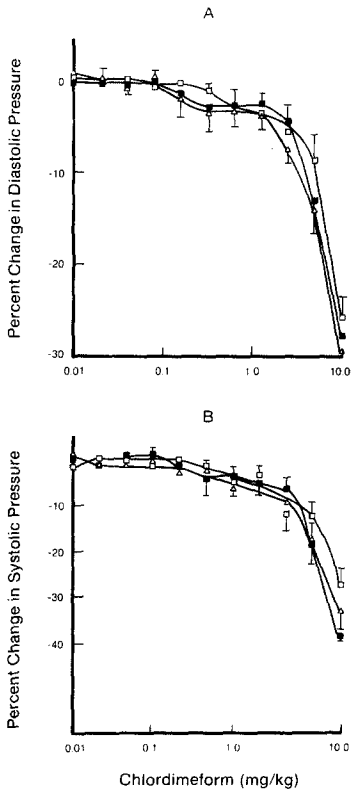

Fig. 2. Reduction in diastolic (A) and systolic (B) blood pressure following i.v. administration of CDF in dogs with no pretreatment (□), pretreatment with 0.5 mg/kg propranolol (■), or 2 mg/kg atropine (▲). Each value is the mean (± SEM) of 5 observations.

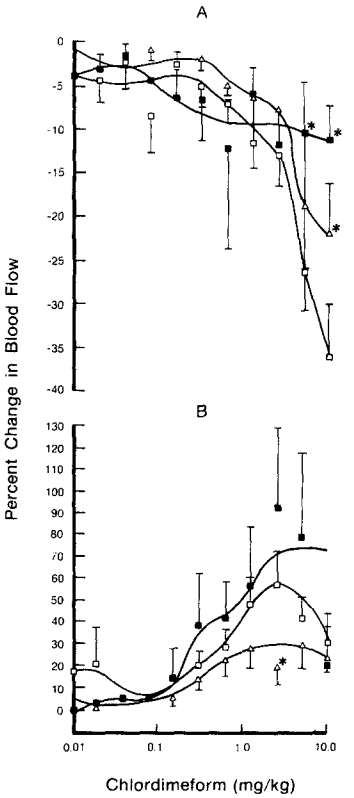

Fig. 3. Initial decrease (A) and subsequent increase (B) in blood flow within femoral artery of dogs following i.v. administration of CDF. Symbols same as in Fig. 2. Each value represents mean (± SEM) of 5 observations. Asterisk (*) indicates significant difference ($P \geq 0.05$) when compared with control value (no pretreatment).

Little or no change was seen in heart rate following the successive administration of eleven increasingly larger doses of CDF except for a modest (6 percent) increase when the last dose (10.2 mg/kg) was administered (Table I). Single doses of 20.5 and 30.0 mg/kg gave a 12.2 percent decrease and a 0.6 percent increase in heart rates, respectively (Table I).

Changes in blood flow within the femoral artery following administration of CDF were gradual in onset and present at relatively low doses. An initial decrease in blood flow was observed when as little as 1.3 mg/kg of CDF was administered (Fig. 3A). Decreases of 26 and 36 percent were noted when the last two injections of the series (5.1 and 10.2 mg/kg) were given. A secondary increase in flow was first observed when 0.32 mg/kg of CDF was given, and a peak response of 56 percent was measured when only 2.6 mg/kg was administered (Fig. 3B). However, when the highest two doses of the series of eleven injections were given, the magnitude of the response declined. Dogs given a single dose of CDF, 20.5 mg/kg exhibited an initial decrease of 62 and a secondary increase of 127 percent in blood flow (Table I). Propranolol blocked the initial reductions in blood flow elicited by 5.1 and 10.2 mg/kg of CDF (the last two injections in the cumulative series) (Fig. 3A), but did not block the secondary increase in flow elicited by CDF (Fig. 3B). Atropine blocked the initial decrease in flow elicited by 10.2 mg/kg CDF (Fig. 3A) and the secondary increase elicited by 2.5 mg/kg CDF (Fig. 3B).

Cardiovascular changes elicited by five different cardiovasoactive agents in the presence of CDF were significantly different from controls (no CDF) in several instances (Figs. 4 and 5). Although CDF reduced the effects of l-norepinephrine on blood flow and heart rate, in neither instance was the difference between control and treated groups significant (Fig. 4). The initial reduction in blood flow following acetylcholine administration was significantly less in the presence of CDF when compared with controls (Fig. 5). A similar observation was made with histamine, except the difference between control and treated groups was not significant (Fig. 5).

A more frequent effect of CDF was the enhancement or potentiation of cardio-vascular changes elicited by the five agents. The increase in systolic pressure following l-norepinephrine administration was significantly higher in the presence of CDF (Fig. 4). Isoproterenol induced changes were larger in every case in the presence of CDF and three of the four changes - reduction in diastolic pressure, increase in heart rate and increase in blood flow - were significantly different when comparing treated with control groups (Fig. 4). Four of five responses elicited by acetylcholine appeared to be augmented in the presence of CDF, but only the secondary increase in blood flow was significantly different when compared with controls (Fig. 5). Mean values for systolic and diastolic pressures, as well as heart rate, were significantly different in the presence of CDF following administration of epinephrine (Fig. 4). The magnitude of the cardiovascular changes elicited by histamine were augmented in four out of five instances in the presence of CDF. However, only the reduction of systolic pressure differed significantly from control values (Fig. 5).

The presence of CDF likewise affected the latency of peak responses elicited by the five agonists in numerous instances (Figs. 4 and 5). Isoproterenol induced changes were affected to the greatest extent. Differences in latent periods between control and

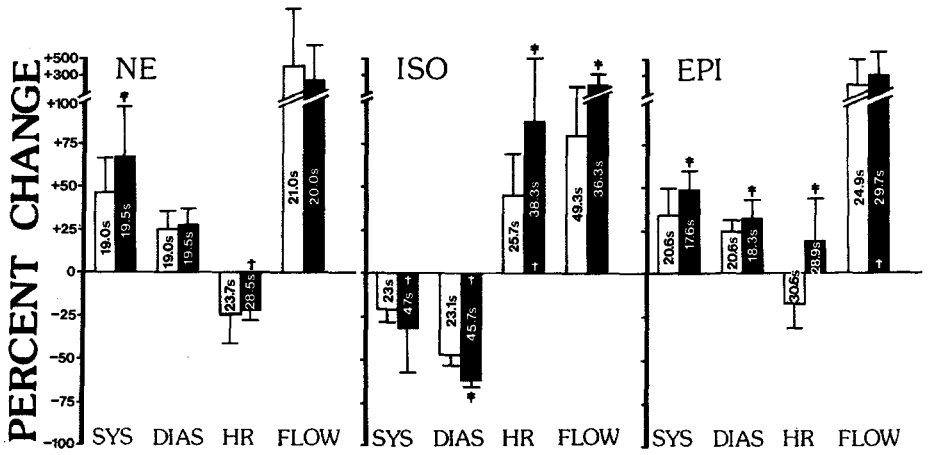

Fig. 4. Effect of 1 ug/kg of l-norepinephrine HCl (NE), 1 ug/kg isoproterenol HCl (ISO), and 2 ug/kg epinephrine bitartrate (EPI) administered i.v. on systolic (SYS) and diastolic (DIAS) blood pressure, heart rate (HR), and blood flow (FLOW). Bars represent mean values (± SEM) of control (clear) and CDF pretreated dogs (shaded). N=8. (∓) and (+) indicate significant differences between control and treated values with respect to magnitude of change and latency of peak response, respectively.

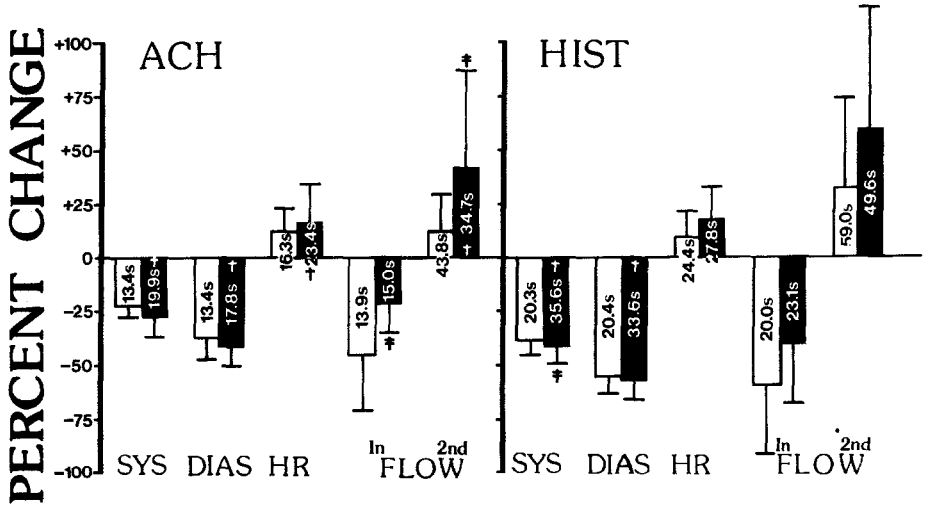

Fig. 5. Effect of 0.5 ug/kg acetylcholine chloride (ACH) and 10 ug/kg histamine dihydrochloride (HIST) administered i.v. on systolic (SYS) and diastolic (DIAS) blood pressure, heart rate (HR), and blood flow (Initial change = In; Secondary = 2nd). Bars represent mean values (± SEM) of control (clear) and CDF pretreated dogs (shaded). N=8. symbols (∓) and (+) same as in Fig. 4.

treated groups were relatively large and were significant in three of the four parameters measured (Fig. 4). Latent periods for acetylcholine induced responses were significantly different in four of the five parameters monitored, although the differences in mean values were relatively small (Fig. 5). Peak depressor responses (systolic and diastolic) elicited by histamine occurred significantly later in the presence of CDF (Fig. 5). The latent periods for heart rate and flow rate were significantly altered in the presence of CDF when norepinephrine and epinephrine, respectively were used as agonists (Fig. 4).

DISCUSSION

Our results show that acute intoxication with CDF could result in cardiovascular collapse. Dose dependent changes in blood pressure were observed when CDF was given to anesthetized dogs in doses ranging from 5.1 to 30 mg/kg. Maximal reductions of 57 and 68 percent for systolic and diastolic pressures, respectively, were seen when 30 mg/kg of CDF was given. Increasing the dose to 50 mg/kg likely would have precipitated a state of severe cardiovascular collapse, as reported earlier (LUND et al. 1978). The reduction in systolic and diastolic pressures, observed 26 seconds post CDF administration, is probably due to a direct cardio-depressant effect. Myocardial depression, as well as a negative chronotropic effect, has been observed in experiments carried out on isolated perfused rabbit hearts in our laboratories (unpublished data) and has been reported by others (LUND et al. 1978). Relaxation of the vasculature could also be contributing to the reduction in systemic pressure.

The initial, transient decrease in blood flow commencing about 27 sec post CDF administration, tends to parallel the reduction in systolic and diastolic pressure (Figs. 2A and B, 3A) and may be a consequence of reduced cardic output. A secondary increase in flow was first observed with doses of CDF (0.32 mg/kg) too small to produce myocardial depression and some 10 to 15 seconds later than initial changes in systemic pressure (Figs. 3B, 1). This may reflect an increased sensitivity of the vasculature to the drug when compared to cardiac tissue. ROBINSON and BITTLE, as noted earlier, reported that demethylchlordimeform, a metabolite of CDF, induces contraction of isolated strips of rabbit central ear artery in concentrations of 10^{-9} to 10^{-4} M. Our results indicate CDF relaxes the vasculature of dogs, reducing peripheral vascular resistance and allowing an increase in blood flow.

Dogs given 30 mg/kg of CDF exhibited a 56.8 and 67.5 percent decrease in systolic and diastolic pressure respectively, and only a 0.6 percent increase in heart rate. The failure to see a compensatory increase in heart rate in the presence of large reductions in systemic pressure may be a consequence of a negative chronotropic effect of CDF on the heart.

Little evidence was obtained in our studies to support the hypothesis that CDF alters cardiovascular parameters by mimicking or blocking adrenergic, cholinergic, or histaminergic receptors. One bit of evidence to the contrary however was the ability of pro-

pranolol to block the initial reduction in blood flow elicited by 5.1 and 10.2 mg/kg of CDF (Fig. 3A). However, propranolol did not block the secondary increase in flow elicited by CDF (Fig. 3B), which is consistent with observations reported by others (LUND et al. 1978). There were two other instances where CDF exerted blocking effects in our studies, i.e. changes in heart rate following administration of epinephrine (Fig. 4) and initial reduction in blood flow following administration of acetylcholine (Fig. 5). In most instances however CDF exerted no blocking effects.

The magnitude of the cardiovascular changes elicited by these five cardiovasoactive agents were augmented in numerous instances, as shown in Figs. 4 and 5. One possible explanation for this, at least with respect to norepinephrine, epinephrine, and isoproterenol is that CDF inhibited MAO and thereby allowed these amines to remain biologically active. As noted previously, it has been shown repeatedly that CDF and other formamidines inhibit MAO in vitro as well as in vivo, resulting in higher levels of tissue amines. More recently, EMRAN, SHANBAKY, and BOROWITZ (1980) reported that CDF and its metabolites can block the release of adrenal catecholamines. Experiments carried out in our laboratories (GHEREZGHIHER 1978) suggest that CDF and other formamidines may act by interfering with uptake of amines, a hypothesis that is compatible with many of the observations made in this study.

A possible explanation for changes in latent periods of cardiovascular responses elicited by the five agonists is that CDF altered the coronary and systemic circulation to such an extent that peak concentrations of agonists occurred at different times. If the circulation had been reduced by CDF, particularly in and around the heart, it seems peak responses might have been delayed in onset, since the agonist would not have reached its site of action as quickly as it did in the absence of CDF. Peak responses were delayed in ten out of eleven instances. The single exception was in the secondary increase in blood flow following acetylcholine administration when the peak response was observed at 43.8 seconds in the absence of CDF and at 34.7 seconds in the presence of CDF.

In conclusion, our results show that CDF is capable of inducing marked cardiovascular changes, if present in sufficient dosage. Our data does not support the hypothesis that CDF interacts with aminergic receptors (adrenergic, cholinergic, or histaminergic), but does show that CDF can potentiate the action of several cardiovasoactive agents. The basic mechanisms whereby this is brought about is unclear, but may be a consequence of inhibition of MAO or altered hemodynamics.

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