

Studies of the Mechanism of Cardiovascular Responses to CI-581

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The mechanism of the biphasic response of arterial pressure to administration of CI-581 was investigated. In dose-response studies of isolated perfused rabbit hearts, contractile tension decreased in direct proportion to dose. A depressor phase only was found in dogs with bilateral vagotomy and bilateral section of the carotid sinus nerve. In cross-circulation studies, acceptor dogs in which only one carotid sinus was exposed to the drug responded with a pressor phase only. In another series of dogs the normal response of decreased arterial pressure to sudden increases of pressure on the carotid sinus was abolished by CI-581. Electroneurograms of the carotid sinus nerve revealed a diminution of frequency response after CI-581. We propose that the depressor phase is due to a direct negative inotropic effect on the myocardium and the pressor phase to a diminution of frequency response of baroreceptors, these being independent and opposing mechanisms and the resultant arterial pressure a summation of the two. Epinephrine-induced arrhythmias in dogs were abolished by CI-581, and the dose-response studies of the functional refractory period of isolated perfused rabbit hearts revealed significant prolongation. This was interpreted to mean that CI-581 has antiarrhythmic properties.

THE ANESTHETIC PROPERTIES of the compound 2-(0-chlorophenyl)-2-methylamino cyclohexanone HCl (CI-581) have been demonstrated in laboratory animals and in man.¹⁻⁷ Corssen observed a pressor response of systolic and diastolic blood pressures as well as an increase in heart rate in man.⁴ McCarthy found a biphasic response of blood pressure in animals.¹

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In using CI-581 clinically as an anesthetic agent we frequently have observed this pressor response; in fewer cases we have found a biphasic response. In the biphasic response, the depressor component comes first, with an onset paralleling loss of consciousness; it is of short duration, usually one to three minutes, and is followed immediately by the pressor response. Experience with CI-581 led us to believe that the drug possessed antiarrhythmic properties. The purpose of this investigation was to examine mechanisms of the effect of CI-581 on the cardiovascular system.

Methods

EFFECTS OF CI-581 ON THE ARTERIAL PRESSURES OF DOGS BEFORE AND AFTER DENERVATION OF BARORECEPTORS

Ten dogs received initial doses of thiamylal 30 mg./kg. in the cephalic vein, and the tracheas were intubated. Anesthesia was maintained with a 66 per cent nitrous oxide and 33 per cent oxygen mixture in a semiclosed circle absorption system; ventilation was controlled with a Bird respirator. Arterial blood gases, maintained at pH 7.35-7.45, P_{CO_2} 35-40 mm. Hg, P_{O_2} 100-150 mm. Hg, were monitored with Instrumentation Laboratories gas analyzers, Model 113 and Model 127. Rectal temperature was monitored with a Yellow Springs Tele-Thermometer Model 43 TD and maintained between 98 and 99° F. The mean femoral arterial blood pressure was measured and recorded utilizing a Statham strain-gauge, Grass Model 7PIA preamplifier, and a Model 7 polygraph.

The ten dogs were divided into three groups with varying baroreceptor reflex capacities produced by surgical denervation. CI-581 5 mg./kg. was administered into the cephalic vein and the effects on mean femoral arterial pressure were determined. Group A, four

TABLE 1. Response of MIFAP to Cl-581 before and after Denervation of Baroreceptors

	Before Denervation						After Denervation					
	After Cl-581, 5 mg./kg.			Control MIFAP (mm. Hg.)			After Cl-581, 5 mg./kg.			Control MIFAP (mm. Hg.)		
	Minimal MIFAP (mm. Hg.)	Duration of Decrease (sec.)	Maximal MIFAP (mm. Hg.)	Duration of Increase (sec.)	Minimal MIFAP (mm. Hg.)	Duration of Decrease (sec.)	Maximal MIFAP (mm. Hg.)	Duration of Increase (sec.)	Minimal MIFAP (mm. Hg.)	Duration of Decrease (sec.)	Maximal MIFAP (mm. Hg.)	Duration of Increase (sec.)
Group A, Bilateral Vagotomy												
Dog 1	160	140	180	600	170	100	190	600	160	120	180	600
Dog 2	125	100	135	1,200	140	70	140	300	120	300	140	0
Dog 3	120	90	140	600	130	70	140	280	120	280	140	300
Dog 4	105	85	145	800	120	105	140	300	105	300	140	450
Mean	125 (± 10)	103.7 (± 25)	150 (± 20)		140 (± 22)	114 (± 37)	152 (± 25)		125 (± 10)	103.7 (± 25)	150 (± 20)	
Group B, Bilateral Division of Carotid Sinus Nerves												
Dog 1	115	90	145	400	130	85	145	100	115	90	145	100
Dog 2	130	95	170	600	100	150	185	60	130	95	170	400
Dog 3	120	100	135	1,100	130	90	130	60	120	100	135	0
Mean	122 (± 8)	95 (± 5)	152 (± 21)		140 (± 17)	108 (± 30)	153 (± 28)		122 (± 8)	95 (± 5)	152 (± 21)	
Group C, Bilateral Vagotomy and Division of Carotid Sinus Nerves												
Dog 1	115	95	145	800	138	100	138	600	115	95	145	0
Dog 2	124	100	135	1,000	140	115	140	300	124	100	135	0
Dog 3	115	110	145	800	145	115	145	1,200	115	110	145	0
Mean	118 (± 5)	102 (± 8)	142 (± 0)		141 (± 1)*	110 (± 0)**	141 (± 1 ***)		118 (± 5)	102 (± 8)	142 (± 0)	

* Significant difference from value before denervation, $P < 0.05$.** Significant difference from value after denervation, $P < 0.025$.

*** No significant difference from control value after denervation.

dogs, received CI-581 before surgical vagal denervation. This group then received bilateral vagotomy at a level immediately caudal to the nodose ganglion and the drug effect was again determined. The intervals between the first and second injections of CI-581 were between 90 and 120 minutes.

Group B, three dogs, received CI-581, 5 mg./kg., before and after the carotid sinus nerve was sectioned bilaterally. The time intervals between the two injections were the same as those for Group A.

Group C, three dogs, received CI-581, 5 mg./kg., after the carotid sinus nerve was sectioned bilaterally and bilateral vagotomy was performed at the same level as in dogs in Group A.

ANTIARRHYTHMIC ACTION OF CI-581

Eight dogs of Groups D and E received an initial dose of thiarnylal 30 mg./kg. administered into the cephalic vein, and endotracheal intubation was accomplished. Anesthesia was maintained with a mixture of oxygen and 1 per cent halothane in a semiclosed circle absorption system; ventilation was controlled with a pressure-cycled respirator. pH and P_{CO_2} were maintained as previously described, but P_{O_2} was 220 to 250 mm. Hg. The EKG was monitored utilizing a Grass Model 7P6A preamplifier and Model 7 polygraph. Mean femoral arterial pressure was measured.

One hour after induction of anesthesia, control cardiac rate, rhythm and mean femoral arterial pressure were determined and epinephrine was administered by a drip technique into the cephalic vein at a rate which was constant in each dog but varied from one experiment to another, ranging from 0.21 to 1.00 μ g./kg./minute (table 2). The rate of administration was recorded by connecting a Grass 7-P58 preamplifier between the infusion bottle of saline solution containing epinephrine and the infusion set, each drop (approximately 0.18 μ g epinephrine) completing an electrical circuit; the signal was recorded by a Grass polygraph simultaneously with the arterial blood pressure and EKG. The epinephrine drip was operated manually; the rate remained constant throughout the experiment.

After ventricular arrhythmias (either multifocal ventricular extrasystoles or ventricular tachycardia) had persisted for approximately ten minutes, CI-581, 6 mg./kg., was administered to the four dogs of Group D as a single dose intravenously in the side opposite that receiving epinephrine, and to the four dogs of Group E by the drip technique at rates ranging from 1.5 to 2.4 mg./kg./min. (table 2). The effects of CI-581 on cardiac rhythm and mean femoral arterial pressure were recorded.

RESPONSE OF THE CAROTID SINUS BARORECEPTOR TO CI-581

Twenty-eight dogs were anesthetized with thiarnylal 30 mg./kg. Anesthesia was maintained with nitrous oxide and oxygen, and blood gases were measured. Three separate groups of experiments were performed utilizing 14 dogs in Group F, which consisted of seven cross-circulation experiments, and seven dogs each in groups G and H.

Group F, Cross-circulation. Bilateral vagotomy and sinus denervation were performed in the donor dog to determine the effect on arterial pressure produced by the drug through mechanisms other than the baroreceptor mechanism. Bilateral vagotomy and right carotid sinus denervation were performed in the acceptor dog so that the drug effect on the perfused left carotid sinus with the sinus nerve intact would be reflected in changes in peripheral arterial pressure.

Donors and acceptors were heparinized, and an *in situ* preparation of the left carotid sinus of the acceptor was perfused with the blood of the donor by a Sigma motor (constant flow) pump superimposed between the two (fig. 1). The total capacity of the polyethylene tubing between the two dogs was 9 ml.; the capacity of the *in situ* preparation was 1 ml.

The mean blood pressures of the external carotid artery of the *in situ* preparation and the femoral arteries of both the acceptor and the donor were measured and recorded. Lead 2 of the EKG of the acceptor was recorded.

CI-581, 5 mg./kg. was administered into the cephalic vein of the donor and the effects on femoral arterial blood pressures of both dogs examined.

Group G, effects of CI-581 on the Responses of the Carotid Sinus Baroreceptors to Changes of Pressure. Seven dogs of Group G were anesthetized with thiamylal. Anesthesia was maintained with nitrous oxide and oxygen. Mean femoral arterial pressure was recorded.

Bilateral vagotomy was performed immediately caudad to the nodose ganglion and the left carotid sinus nerve was divided so that the only baroreceptor fibers intact would be those of the right carotid sinus nerve. The animals were heparinized, the right common carotid artery divided and the ends reapproximated over a T-tube with the side arm leading to a 500-ml. reservoir partially filled with 0.9 N saline solution. The side arm was clamped until the test began. All branches of the common carotid artery except the internal carotid artery were ligated.

The internal carotid artery was divided, the distal end ligated, and the proximal end anastomosed to a polyethylene tube 2 cm. in length. The distal end of the tube was anastomosed to the external jugular vein so that the internal carotid artery could be occluded by clamping the tube, thus avoiding mechanical pressure on the arterial wall at the time of testing. At the test the common carotid ar-

tery was clamped, measured pressure was applied to the reservoir bottle, the internal carotid artery anastomosis was occluded at the level of the polyethylene tube, the clamp on the side arm of the T-tube was released, and the changes in arterial pressure produced by varying the pressure on the carotid sinus from 0 to 150 mm. Hg were observed.

After this observation, the internal carotid anastomosis was unclamped and the side arm of the T-tube closed; an interval of 30 minutes elapsed before further testing. CI-581, 2 mg./kg., was then administered into the cephalic vein of one dog; 5 mg./kg. to three dogs; and 10 mg./kg. to three others. The test was repeated after five minutes, and again after 15 minutes to evaluate recovery.

Group H, Recording of Sinus Nerve Action Potential. A bypass of the common carotid artery was made in the seven dogs of Group H and the pressure controlled in the manner described. In these preparations, however, the internal carotid artery was not anastomosed or clamped. The carotid sinus nerve was dissected free and divided near its junction with the glossopharyngeal nerve, the distal section of the nerve placed on a pair of platinum electrodes approximately 2 mm. apart in a liquid

TABLE 2. Effects of CI-581 on Epinephrine-induced Arrhythmias*

	Control MFAP (mm. Hg)	Rate of Epinephrine Drip µg./kg./ min. ²²	After Epinephrine		After CI-581			
			MFAP (mm. Hg)	Time (sec.) until Arrhyth- mias	Dose	MFAP (mm. Hg)	Time (sec.) until Normal Rhythm	Duration of Normal Rhythm
Group D, Single Injection								***
Dog 1	110	0.53	168	65	6 mg./kg.	140	<20	30 sec.
Dog 2	150	0.30	190	60	6 mg./kg.	180	<20	25 sec.
Dog 3	145	0.26	170	90	6 mg./kg.	130	<20	65 sec.
Dog 4	120	0.21	140	180	6 mg./kg.	100	<20	90 sec.
Group E, Drip Technique								
Dog 1	120	0.29	140	120	2.4 mg./kg./min.	130	240	7 min.
Dog 2	112	0.30	164	90	2.4 mg./kg./min.	150	40	6 min.
Dog 3	85	1.0	130	90	1.5 mg./kg./min.	135	120	5 min.
Dog 4	132	1.0	162	120	2.0 mg./kg./min.	155	90	5 min.

* Dogs anesthetized with oxygen and halothane 1 percent.

** After arrhythmias occurred epinephrine was continued at the same rate until the experiment was terminated.

*** Normal rhythm was still present in all experiments when terminated.

paraffin layer. Spontaneous action potentials of the carotid sinus nerve were amplified with a Tektronix RM122 preamplifier, displayed on the screen of a cathode-ray storage oscilloscope, and photographed simultaneously with external carotid artery pressure.

The sinus nerve has two types of impulses: one of large amplitude, thought to result from the rate of change of the systolic phase of the pulsatile pressure; the other small and seen during the diastolic phase. The maximal sensitivity of this reflex mechanism exists between pressures of 85–110 mm. Hg.^{13–15}

The frequency response of the sinus nerve impulse to spontaneous pulsatile pressure was recorded before and at intervals of three, five, ten and 25 minutes after administration of CI-581, 6 mg./kg., intravenously. The frequency response to controlled nonpulsatile pressure progressively increasing from 50 to 150 mm. Hg was also ascertained before and after administration of the drug at intervals of five, 15, and 20 minutes. Responses to constant nonpulsatile pressures of 100 and 150 mm. Hg were recorded before and five and 15 minutes after drug administration.

ISOLATED HEART STUDIES

Isolated rabbit hearts were perfused with a solution of 7.0 Gm. sodium chloride, 0.42 Gm. potassium chloride, 0.24 Gm. calcium chloride, 2.10 Gm. sodium bicarbonate, 0.143 Gm. monobasic sodium phosphate, 0.20 Gm. magnesium chloride, 2.0 Gm. dextrose, and 45 Gm. sucrose in distilled, demineralized water, to a total volume of 1,000 ml. The solution was saturated with 95 per cent oxygen and 5 per cent carbon dioxide. pH values remained within the range of 7.35 to 7.45 and temperatures were maintained constant at 38° C. The pressure required for good coronary perfusion, approximately 45 to 55 cm. water, was kept constant by means of a modified Anderson-Craver Pyrex coronary perfusion apparatus.^{6,9}

Myocardial contractile tension was monitored by placing a suture in the apex of the ventricle and attaching it to an FT-03B Grass force-displacement transducer, calibrated with one gram of weight. Surface electrical phenomena were monitored as bipolar surface electrograms recorded with a Grass Model 5 polygraph.^{10,11} Surface electrical phenomena

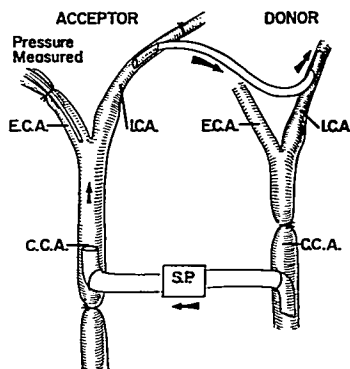


FIG. 1. In situ carotid sinus cross-circulation. C.C.A. = common carotid artery; E.C.A. = external carotid artery; I.C.A. = internal carotid artery; S.P. = sigma motor pump.

and contractile tension were studied in nine preparations.

Measurement of the functional refractory period was accomplished by the twin-pulse method of Rosenbluth.¹² The stimulating electrodes were 26-gauge wire needles one-half inch long, inserted into the myocardium approximately 1 cm. apart on the posterior side at the uppermost part of the ventricular septum. After the electrodes were in place the area of the sinus node was removed. The hearts were paced with a Grass SD-5 stimulator, utilizing 40 volts of 1-millisecond duration at a rate slightly greater than normal, 140–200/minute, kept constant throughout each experiment. The test stimulus was identical to and was applied through the same electrode as the drive stimulus. The delay between the drive stimulus and the second pulse, the test stimulus, was gradually increased until an extrasystole was observed. The shortest period of delay between the twin pulses was recorded as the functional refractory period. The refractory periods were measured in eight preparations different from those used for studies of surface electrical phenomena and contractile tension.

Each preparation was subjected to the normal perfusate and control values obtained.

CI-581 was then added to the perfusate, the concentrations increased, and the effects examined at 10-minute intervals. CI-581 concentrations ranging from pD 4.3 to pD 3.4 were selected because pD CI-581 4.3 was calculated to be a minimal anesthetic dose and pD CI-581 3.4 was the concentration that consistently and completely abolished the development of contractile tension.

Results

RESPONSES MFAP TO CI-581 BEFORE AND AFTER SURGICAL DENERVATION OF THE BARORECEPTORS

The typical responses of the mean femoral arterial pressure (MFAP) to intravenous administration of CI-581, 5 mg./kg., are shown in figure 2. The variations in MFAP seen were consistent in all experiments (table 1).

Group A. A biphasic response of the MFAP was produced in all dogs by CI-581, 5 mg./kg., before bilateral vagotomy. The initial component was a depressor phase, with a mean decrease of 21 mm. Hg, range 10 to 30

mm. Hg. Duration was highly variable, ranging from 20 to 200 sec. The depressor phase was followed immediately by a pressor phase with a mean increase of maximal pressure 25 mm. Hg above the control value, range 10 to 40 mm. Hg. The duration of the pressor phase was greater than that of the depressor in all dogs, with variability ranging from 500 to 1,200 sec.

After bilateral vagotomy the mean control MFAP was 15 mm. Hg greater than before section, indicating that some baroreceptor activity had been removed. CI-581, 5 mg./kg., again produced a biphasic response of MFAP, with an initial depressor phase with a mean value of 26 mm. Hg, range 10 to 60 mm. Hg. The duration was again variable from 60 to 300 seconds, but in each dog it was greater than before bilateral vagotomy.

The depressor phase was followed immediately by a mean increase in the MFAP of 12 mm. Hg. Dog 2 of this group did not respond with a pressure greater than the control level; after 300 seconds the depressor

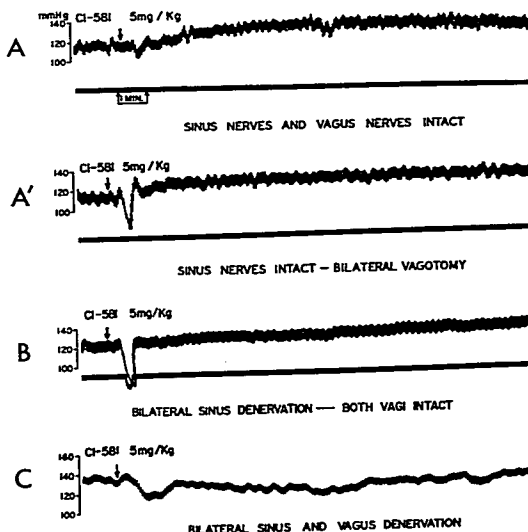


FIG. 2. Response of mean femoral arterial pressure to CI-581, 5 mg./kg., in dogs anesthetized with nitrous oxide-oxygen. A = normal dogs; very slight depressor phase followed by pressor phase; A' = after bilateral vagotomy; biphasic response; B = vagi intact but with bilateral division of the carotid sinus nerve, biphasic response; C = bilateral vagotomy and bilateral division of the carotid sinus nerve, only a depressor phase, which did not completely recover until after 15 minutes.

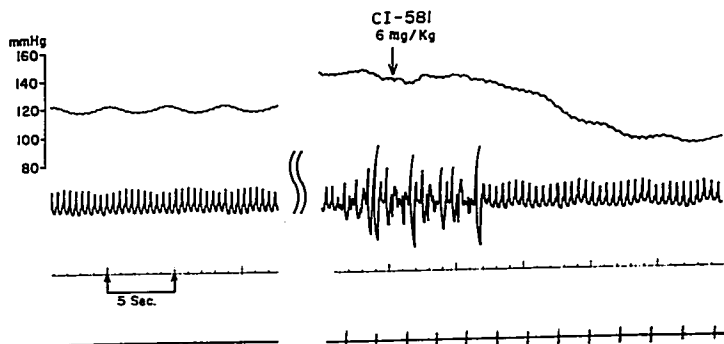


FIG. 3. Antiarhythmic effect of CI-581. Ventricular arrhythmia produced by epinephrine drip $0.22 \mu\text{g./kg./min.}$ in dog anesthetized with halothane 1 per cent and oxygen. Tracings from top to bottom are: 1. mean femoral arterial pressure; 2. EKG, lead 2; 3. time; 4. epinephrine drip, $0.18 \mu\text{g./drop.}$ Intravenous administration of CI-581 6 mg./kg. completely abolished the ventricular arrhythmia.

phase was gone and MFAP returned to control level. In dogs that did exhibit the pressor phase, the duration was again variable, ranging from 300 to 600 seconds, and in all dogs the duration of the pressor phase was less after bilateral vagotomy than before.

Group B. The MFAP of the control dogs responded to CI-581, 5 mg./kg. , in a fashion similar to MFAP in control dogs of Group A before section (table 1). Again, there was a biphasic response, with a mean decrease of MFAP of 27 mm. Hg followed by a mean increase of 30 mm. Hg greater than control values. Again, the durations of both phases were variable, but the pressor phase was longer than the depressor phase. After right and left carotid sinus denervation the mean value of the pressures was 18 mm. Hg greater than the mean value of control pressures before section. All animals responded with a depressor phase, with a mean of 32 mm. Hg . This was followed by a pressor phase in two dogs; MFAP of one returned to the control level without a pressor response. Mean pressor response was 13 mm. Hg . Duration was again variable, ranging from 0 to 400 seconds and was shorter than that before denervation.

Group C. The response to CI-581, 5 mg./kg. , before section was the same as that in the

dogs of Groups A and B before section. The response was again biphasic. The mean decrease of MFAP was 16 mm. Hg ; the increase was 24 mm. greater than in the controls. The pressor phase lasted longer than the depressor phase in all dogs.

After bilateral total denervation, MFAP was 23 mm. Hg higher than the mean control value. CI-581, 5 mg./kg. , produced only a depressor phase. The difference between the mean of the control values and the minimal MFAP after CI-581 was 31 mm. Hg . Durations of depressor phases were variable but greater than before denervation. The mean durations of the biphasic components of the MFAP of all ten dogs of this experiment were determined before denervation. The mean duration of the depressor phase was 1.2 minutes ; duration of the pressor phase 13.5 minutes .

ANTIARRHYTHMIC ACTION OF CI-581

A typical response of dogs with epinephrine-induced arrhythmias to an injection of CI-581, 6 mg./kg. is shown in figure 3, and the responses to single injections and to infusion of CI-581 are shown in table 2.

Arrhythmias occurred in all dogs anesthetized with halothane and receiving epineph-

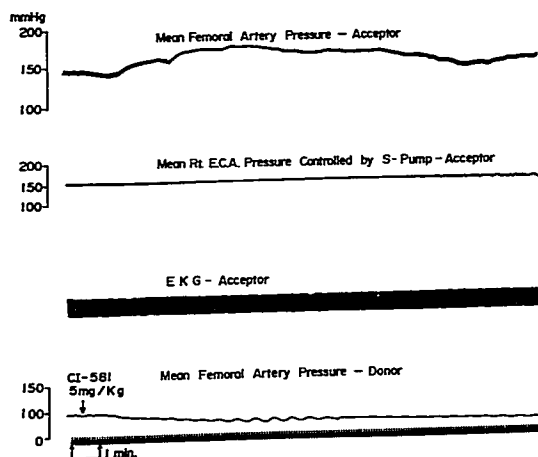


FIG. 4. Cross-circulation experiments demonstrating the response of arterial pressure to exposure of the carotid sinus to CI-581. The donor dog had bilateral vagotomy and bilateral division of the carotid sinus nerve; the acceptor had bilateral vagotomy and division of the left carotid sinus nerve. (See circulation diagram, Fig. 1.)

rine. Onset ranged from one to three minutes after the infusion began. After a single injection of CI-581, 6 mg./kg., all dogs of Group D responded with conversion to normal rhythm within 20 seconds. The duration of normal rhythm was short, ranging from 25 to 90 seconds.

With the infusion technique all dogs responded with conversion to normal rhythm, but the times between drug administration and appearance of normal rhythm were greater than in dogs of Group D. Once normal rhythm occurred, it remained as long as the infusion continued. The MFAP was elevated in all dogs after epinephrine infusion began, and depressed after the administration of CI-581 in all dogs except in dog 3 of Group E. Although CI-581 produced decreases of MFAP when compared with MFAP before the administration of epinephrine, the pressures were still above the initial values in six of the eight dogs. The MFAP values of dogs 3 and 4 of Group D were depressed 10 and 16 per cent, respectively, when compared with pre-epinephrine levels.

RESPONSE OF CAROTID SINUS BARORECEPTORS TO CI-581

Group F. The responses of the MFAP values of the donor and acceptor dogs to CI-

581, 5 mg./kg., shown in figure 4, are typical of the data of this series (table 3).

After administration of CI-581 to the donors, all acceptors responded with increases in MFAP. The difference in the mean values of MFAP before and after receiving CI-581 was 26.2 mm. Hg, which was significant ($P < 0.01$), representing an 18 per cent increase over the control pressure. The mean duration of the pressor responses was 14 minutes. A depressor response was not observed in any acceptor dog.

All donor dogs responded with depressor phases. The difference in mean MFAP values before and after CI-581 was 22 mm. Hg, also highly significant ($P < 0.005$), representing a 19 per cent decrease from the control pressure. The mean duration of the depressor phases was seven minutes. A pressor response was not observed any donor dog.

Group G. The responses of MFAP of sudden changes of pressure in the carotid artery before and after the intravenous administration of CI-581 are presented in table 4. A 35 per cent mean decrease of MFAP was produced by changes in external carotid artery pressure, within the normal range of the Blutdruck-Charakteristik curves described by Koch in 1931.¹⁶

After administration of CI-581, a Blutdruck-Charakteristik plot revealed a shift of the curves to the right in all dogs; in other words, the usual fall of the MFAP produced by varying the pressure on the carotid sinus was diminished by the administration of CI-581. The depression of MFAP was inversely proportioned to the dose. One dog receiving CI-581, 2 mg./kg., had a 20 per cent reduction in the reflex capacity of the carotid sinus; three dogs receiving 5 mg./kg. had 50 to 60 per cent reduction; three dogs receiving 10 mg./kg. had 80 to 100 per cent reductions.

Fifteen minutes after administration of CI-581 there was partial recovery of reflex activity of the carotid sinus in six of the dogs. At this time, dog 3 had completely recovered and responded with 27 per cent more activity than had been present before administration of the drug.

Group H. In all dogs, CI-581, 6 mg./kg., diminished the frequencies of both large- and small-amplitude action potentials produced by pulsatile pressures. Typical electroneurograms are shown in figure 5. CI-581 also diminished the frequencies of both types of potentials that occurred with progressively increasing nonpulsatile pressures from 50 to 150 mm. Hg. The frequency responses of both types to constant nonpulsatile pressures were also diminished.

In all dogs, the frequency responses to pulsatile, constant nonpulsatile, and progressively-increasing nonpulsatile pressures had diminished five minutes after the drug was administered, and in all animals there was partial recovery in 15 minutes.

EFFECTS OF CI-581 ON THE ISOLATED HEART

The mean changes in contractile amplitude, functional refractory period and electrical heart rate obtained with varying concentrations of CI-581 (table 5) indicated that when CI-581 was added to the perfusate in concentrations ranging from 5.06×10^{-5} M to 3.54×10^{-4} M significant negative inotropic effects were produced, as well as negative chronotropic effects and prolongation of functional refractory periods at all doses, with the exception of contractile tension at the lowest dose ($P < 0.01$).

Degree of change was directly proportional

TABLE 3. Effects of CI-581 5 mg./kg. on Mean Femoral Arterial Pressure in Cross-Circulation Experiments

Experiment No.	Acceptor Dogs (left carotid sinus nerve divided and bilateral vagotomy)				Donor Dogs (right and left carotid sinus nerves divided and bilateral vagotomy)			
	Control MFAP (mm. Hg.)	MFAP after CI-581 (mm. Hg.)	Duration of Effect (min.)	Percentage Change MFAP	Control MFAP (mm. Hg.)	MFAP after CI-581 (mm. Hg.)	Duration of Effect (min.)	Percentage Change MFAP
1	150	175	20	+10.0	100	50	17.00	-50.0
2	182	203	15	+11.5	98	05	0.7	-33.6
3	143	100	12	+11.0	138	110	1.3	-13.7
4	160	100	13	+18.8	180	155	5.0	-13.9
5	130	175	10	+34.6	142	135	0.6	-4.9
6	100	177	18	+10.6	125	110	15.0	-12.0
7	114	142	9	+24.6	180	175	1.0	-2.7
Mean	148.4 (± 22.2)	174.0 (± 10.7)*	13.0 (± 4.1)	+18.4 (± 8.7)	137.6 (± 33.0)	115.0 (± 45.4)**	6.7 (± 6.7)	-18.0 (± 10.8)

* Significantly different from control, $P < 0.01$.

** Significantly different from control $P < 0.005$.

TABLE 4. Effect of CI-581 on Response of Mean Femoral Arterial Pressure Produced by Changes in External Carotid Artery Pressure

Dog	Dose of CI-581 (mg./kg.)	Control			5 Minutes after CI-581			15 Minutes after CI-581		
		MFAP (mm. Hg.) when ECAP* = 0	MFAP (mm. Hg.) when ECAP = 150 mm. Hg.	Per cent Decrease in MFAP**	MFAP (mm. Hg.) when ECAP = 0	MFAP (mm. Hg.) when ECAP = 150 mm. Hg.	Per cent Decrease in MFAP**	MFAP (mm. Hg.) when ECAP = 0	MFAP (mm. Hg.) when ECAP = 150 mm. Hg.	Per cent Decrease in MFAP**
1	2	157	98	37.6	143	100	30.1	148	98	33.8
2	5	155	110	29.0	142	115	11.9	150	110	26.7
3	5	137	100	27.0	110	98	11.0	140	92	34.3
4	5	188	112	50.0	200	150	25.0	187	120	35.8
5	10	139	100	28.1	144	144	0.0	137	100	27.0
6	10	147	90	38.8	90	85	5.5	150	108	28.0
7	10	140	92	34.3	145	135	6.9	142	105	26.1

* ECAP = external carotid artery pressure.

** Per cent difference in MFAP at minimum and maximum ECAP values.

to concentration. Fifty per cent of maximal changes of both functional refractory period and negative chronotropic effect occurred very near a concentration of 1.52×10^{-4} M. The concentration which produced 100 per cent depression of contractile tension produced 37 per cent depression of the electrical heart rate. Thus, the drug appeared to uncouple electrical and mechanical activity.

Discussion

These results indicate that CI-581 possesses antiarrhythmic properties, produces a direct negative inotropic effect on the myocardium and diminishes the frequency response of the carotid sinus baroreceptors.

The antiarrhythmic property demonstrated by reversal of epinephrine-induced ventricular arrhythmias in dogs anesthetized with halothane might be questioned as being due to the concomitant fall in blood pressure. We believe the antiarrhythmic property is a quinine-like phenomenon not directly related to blood pressure changes. This is supported by the increase in functional refractory period; furthermore, fall in blood pressure was not dramatic. Only two of eight dogs responded with pressures less than those present before administration of epinephrine.

The depression of MFAP following administration of CI-581 to the dogs receiving epinephrine must have been due at least in part to a negative inotropic effect on the myocardium. Although we did not examine the pos-

sibility of alpha-receptor blocking properties, we thought it unnecessary to do so because the pressor response following the depressor phase was the result of a diminution of the frequency response of the baroreceptors. It should be noted that the efferent limb of this reflex involves sympathetic discharge initiated from the medullary vasomotor centers.

The depressor phase of MFAP occurred in all intact dogs receiving CI-581, not just those pretreated with epinephrine. This was due at least partially to a direct negative inotropic effect on the myocardium. This was suggested by the failure of the acceptor dogs of the cross-circulation series to respond to CI-581 with a depressor phase and by the decreased contractile tension seen in the isolated hearts. By examining the dose-response data (table 5) of the isolated hearts it can be seen that the drug concentrations which produced 100 per cent depression of the contractile tension of the ventricle produced only a 37 per cent depression of the rate of electrical excitation. Therefore, it appears that in higher dose ranges CI-581 is capable of uncoupling the excitation-contraction process. These findings suggest that if a dose severely depressant to the heart were administered clinically, the heart would be in standstill for a period of time with a fairly normal EKG pattern.

We conclude that the pressor phase of MFAP that occurs after administration of CI-581 is a result of a direct effect on the baroreceptor mechanism, producing a diminution

of frequency response. This was suggested by the results of studies in dogs with partial and complete denervation of the baroreceptors. Here the MFAP values of all dogs responded to CI-581 in a biphasic manner before section. Dogs with partial denervation of the baroreceptors also had biphasic responses, but these were not statistically different from control values. However, the number of experiments, only four and three in each group, limited the value of negative results of such analysis.

In the group with complete denervation, baseline pressure was statistically higher than control pressure before section even though only three experiments were done ($P < 0.05$). MFAP responded to CI-581 with a depressor phase significantly different from the post-denervation value ($P < 0.025$); the duration of the depressor phase was much longer in all dogs, and MFAP returned to the post-denervation level without a pressor response. This could mean that the pressor response to CI-581 was due to diminution of the sensitivity of the baroreceptors independent of the depressor phase, or was a normal baroreceptor reflex response to the depressor phase. This was clarified by the cross-circulation experiments. When CI-581 was administered to the donor dogs, the acceptors responded with only pressor phases significantly different from the control values, suggesting that the drug had diminished the activity of the carotid sinus baroreceptors. The donor dogs which were also denervated responded as in the previous denervated series, with only depressor phases statistically different from control values. This again demonstrated that the baroreceptors must be intact for the pressor phase to occur.

The diminished response of the baroreceptors to CI-581 could have been the result of a direct effect on the baroreceptors, or secondary to smooth-muscle relaxation of the carotid arterial wall with failure to activate the baroreceptors. Results of the cross-circulation series suggest that the latter was not the case, because the pressure within the *in situ* preparation was held constant; to demonstrate this in another way, the responses of MFAP to sudden changes in pressure on the carotid sinus before and after CI-581 were examined. The decreased response of the MFAP to

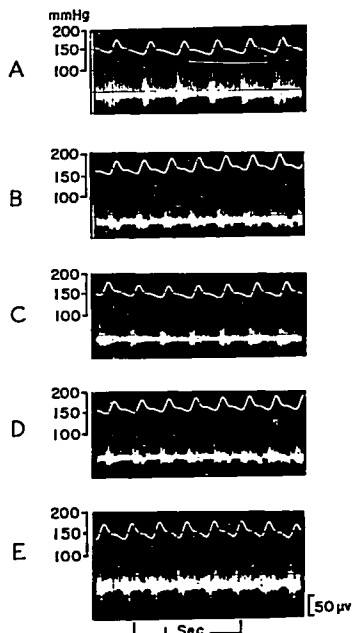


FIG. 5. Effect of CI-581 on the frequency response of baroreceptor fibers of sinus nerve to pulsatile pressure. Pulsatile pressure of the external carotid artery (upper tracing) and electro-neurogram of the sinus nerve (lower tracing) demonstrating the depression of the frequency of the response of the baroreceptor impulses to a pulsatile pressure after the intravenous administration of CI-581. A = before CI-581; B = 3 minutes after CI-581; C = 5 minutes after CI-581; D = 10 minutes after CI-581; E = 25 minutes after CI-581.

changes of pressure suggested that the pressor response which occurred after CI-581 resulted from decreased sensitivity of the baroreceptors. The electro-neurograms of the carotid sinus nerve demonstrated that this interpretation was correct. The electro-neurograms, not being from single fibers, did not permit the frequency response from the carotid sinus nerve to be quantitated accurately, but they definitely demonstrated that a change occurred. A gross estimate was made that a

Percent Change in Functional Reflexory Period	Percent Change in Contractile Tension	Percent Change in Electrical Rate	Number of Experi- ments***	Concentration of CI-581				
				$6.00 \times 10^{-4} \text{ M}$ pD = 4.30103	$1.01 \times 10^{-3} \text{ M}$ pD = 3.99561	$1.02 \times 10^{-3} \text{ M}$ pD = 3.81823	$2.02 \times 10^{-3} \text{ M}$ pD = 3.69101	$2.05 \times 10^{-3} \text{ M}$ pD = 3.59000
8	$14.0^{**} \pm 4.0$	31.0 ± 2.8	8	18.0 ± 3.7	67.4 ± 6.1	86	86.1 ± 3.7	108.8 ± 6.2
0	8.8 ± 10.2	30.1 ± 4.3	0	61.2 ± 4.5	71.3 ± 4.0	80	95.4 ± 2.8	142.5 ± 1.1
0	7.6 ± 1.0	14.0 ± 1.5	0	20.3 ± 5.4	28.2 ± 2.3	30.6	32.8 ± 2.0	37.0 ± 0.7

* pD = negative log of dose.

** Mean with SEM.

*** Each experiment is the mean of three observations.

Note: All changes were significant ($P < 0.01$) except the changes in contractile tension at the lowest dose.

40-50 per cent diminution of the frequency response occurred after CI-581. The carotid sinus nerve was chosen because the baroreceptor fibers there are somewhat more sensitive to unit changes in blood pressure than the aortic baroreceptors¹⁴; besides, the aorta is not suitable for this type of experimentation.

Although the durations of the components of the biphasic response were variable, when dogs were studied before denervation the mean depressor phase was 1.2 minutes; pressor phase, 13.5 minutes. This is comparable to what has been seen clinically.

Summary

We propose that one mechanism of the pressor response seen after administration of CI-581 is reflex resulting from the depression of the frequency response of the baroreceptor fibers, and that this is probably due to the failure of the generator potential. The myocardial effects comprise an antiarrhythmic and a negative inotropic component. We propose that the depressor phase of the arterial blood pressure, occasionally found in patients and seen in the dogs in this experiment, is due at least in part to the negative inotropic effect on the myocardium. The occasional clinical manifestation probably is due to the small magnitude, short duration and summation with the pressor effect.

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