Table VI—Analysis of Variance of Table V Data

Source	Degrees of Freedom	Mean Square	*F*-Value	Significance at 5% Level
Drug Extracted from Urine				
Dose	2	48.5496		
Days	2	0.0384	1.59	N.S.
Dose × days	4	0.0242		
Drug Extracted from Water				
Dose	2	0.5269		
Days	2	0.0757	1.05	N.S.
Dose × days	4	0.0721		

where a = intercept, b = slope, σ^2 = mean square of error, x = dose, and $\bar{x}$ = mean dose.

The percent error in terms of concentrations for a given response is obtained by

$$\text{percent error} = \frac{\sqrt{\text{variation } (\hat{x})} \cdot 10}{x} \qquad \text{(Eq. 6)}$$

Therefore, for a drug concentration of 0.5 mcg./ml. in plasma, the error would be estimated as 1.99%; and for a drug concentration of 10 mcg./ml. in urine, it would be 2.23%.

Effect of Storage at 4° in Whole Blood, Plasma, and Serum—Sotalol·HCl was extracted and analyzed from the plasma prepared from spiked whole blood on 2 subsequent days. The drug was extracted and analyzed from spiked plasma and serum on 2 subsequent days and on the 6th day after spiking.

The data obtained are listed in Table III. The analysis of variance is given in Table IV.

The results indicate that there is no contribution of the different media to the error among days, *i.e.*, there is no difference in the spectrofluorometric response whether the medium spiked was whole blood, plasma, or serum.

Effect of Storage at 4° in Water and Urine—Sotalol·HCl was extracted from spiked water and urine on 3 subsequent days and analyzed. The data are listed in Table V.

Since there were no duplicate determinations, the dose × day-interaction term was used as the error term in the analysis of variance (Table VI). No difference was found among days when the drug was stored in urine or water.

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Vasomotor and α-Block Negating Actions of a Hydroxamic Acid

HORST KEHL

Abstract □ β-Hydroxylamine cinnamonyl hydroxamic acid produced a rapidly reversible hypotension in the anesthetized dog. The hypotensive action of β-hydroxylamine cinnamonyl hydroxamic acid was not augmented by α-receptor block, nor was it negated by β-receptor block. In the presence of an α-blocking agent, the classical vasodepressor action of epinephrine was clearly reversed by the simultaneous intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid. The β-hydroxylamine cinnamonyl hydroxamic acid-induced hypotension and α-block negation could not be explained in terms of any known neurohumoral mediator mechanism; its action appears to be direct and attributable to its unique molecular structure.

Keyphrases □ Hydroxamic acid derivative—vasomotor, α-block negating action □ β-Hydroxylamine cinnamonyl hydroxamic acid—hypotensive, α-block negating action □ α,β-Receptors, blockade effect—β-hydroxylamine cinnamonyl hydroxamic acid action □ Antihistamine effect—β-hydroxylamine cinnamonyl hydroxamic acid action

Hydroxamic acids may be described as *N*-hydroxylated amides occurring naturally in plants and microbes. Neilands (1) described the outstanding biochemical features of hydroxamic acids and reviewed their biological actions. Because phenylethyl hydroxamic acids (Ar—C—C—CO—NH—OH), analogous to vasoactive phenylethylamines, have never been examined for vasomotor activity, a number of hydroxamic acids with two- and three-carbon structures juxtapositioned between a phenyl ring and the hydroxamic acid moiety

Table I—Cardiac and Vasodepressor Responses to Incremental Intravenous Bolus Doses of β-Hydroxylamine Cinnamonyl Hydroxamic Acid

β-Hydroxylamine cinnamonyl hydroxamic acid bolus dose, mg./kg.	0.3 $N = 9$	0.6 $N = 10$	0.9 $N = 8$	1.2 $N = 10$	1.5 $N = 11$	1.8 $N = 7$	2.1 $N = 6$
Systolic pressure reduction (mean ± *SE*), mm. Hg	23.7 ± 1.4	39.7 ± 2.2	57.1 ± 2.0	64.5 ± 1.5	76.3 ± 1.8	92.4 ± 2.4	96.5 ± 2.5
Diastolic pressure reduction (mean ± *SE*), mm. Hg	17.5 ± 1.2	27.6 ± 1.2	40.8 ± 1.6	45.6 ± 1.6	48.5 ± 1.3	53.3 ± 2.1	57.5 ± 1.3
Heart rate[a], beats/min.							
Control	138 ± 1.5	131 ± 1.5					
Experimental	141 N.S.	161 p 0.05					

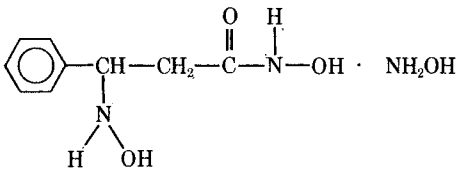
[a] Heart rate was recorded as the mean of the means of 9 dogs at the 0.3-mg./kg. dose level, and of 10 dogs at the 0.6-mg./kg. dose level. Control heart rate was recorded for 1-min. periods over a 10-min. interval prior to the bolus injection of β-hydroxylamine cinnamonyl hydroxamic acid. Experimental heart rate was measured at peak response after bolus injection and statistically evaluated by comparison with the control mean.

(Ar—$C_{2,3}$—CO—NH—OH) were synthesized and studied pharmacologically in this laboratory to examine structure–activity relationships (2).

β-Hydroxylamine cinnamonyl hydroxamic acid (Ar—CNOH—C—CO—NHOH) was observed to produce a rapidly reversible, yet well-controlled, dose-related hypotension in anesthetized dogs (3). This compound was of particular interest because of its special analogy with numerous vasoactive amines, its vasodepressor action, and its ability to negate an α-adrenergic block. These interesting pharmacodynamic actions of β-hydroxylamine cinnamonyl hydroxamic acid may be related to the unique electron density of the terminal nitrogen in the hydroxamate radical.

EXPERIMENTAL

Synthesis of β-Hydroxylamine Cinnamonyl Hydroxamic Acid—β-Hydroxylamine cinnamonyl hydroxamic acid was synthesized and prepared by the general method of Cooley *et al.* (4). The white powdery precipitate produced by the addition of ethylcinnamate to an alkaline methanolic hydroxylamine solution was purified in crystalline form from a 1:1 mixture of heptane–ethanol. Elemental, radical, and IR analyses established the molecular structure (I) of β-hydroxylamine cinnamonyl hydroxamic acid[1].



I

All physical characteristics of β-hydroxylamine cinnamonyl hydroxamic acid agreed well with those initially described by Possner (5). A positive $FeCl_3$ test for the presence of hydroxamic acid moiety and periodic IR analysis showed β-hydroxylamine cinnamonyl hydroxamic acid to be stable for approximately 30 months. β-Hydroxylamine cinnamonyl hydroxamic acid was soluble to the extent of 2.4 g. in 100 ml. water and was prepared for parenteral administration in isotonic saline.

Procedures—Mongrel dogs of both sexes, weighing 13–18 kg., were anesthetized with pentobarbital sodium (30 mg./kg. i.v.). A femoral artery was cannulated, and the systemic blood pressure was recorded with a Statham pressure transducer and Grass model 5 polygraph. Quantitative evaluation of blood pressure was achieved by internal calibration of the polygraph and checked against direct manometric readings. Respiration was recorded with a conventional pneumograph.

All drugs were administered intravenously through a polyethylene catheter inserted into the saphenous vein of a contralateral limb. Continuous infusions of β-hydroxylamine cinnamonyl hydroxamic acid at precise flow rates were made with an Aminco peristaltic pump and Manostat flowmeter. β-Hydroxylamine cinnamonyl hydroxamic acid was prepared for intravenous administration in concentrations expressed as the acid in isotonic saline; this preparation was neutral in pH.

Since the vasodepressor response to a bolus intravenous injection of β-hydroxylamine cinnamonyl hydroxamic acid was prompt and rapidly reversible, all experiments—except dose–response studies—were designed to elucidate the mechanism of action of β-hydroxylamine cinnamonyl hydroxamic acid under the condition of a standard, sustained, intravenous infusion (0.6 mg./kg./min.).

RESULTS

Cardiovascular Responses to β-Hydroxylamine Cinnamonyl Hydroxamic Acid—In 61 experiments with 20 dogs, the cardiovascular responses to progressively increasing incremental doses of β-hydroxylamine cinnamonyl hydroxamic acid were studied. An intravenous bolus injection of 0.3 mg./kg. β-hydroxylamine cinnamonyl hydroxamic acid produced a decrease in mean systolic blood pressure of about 24 mm. Hg at peak response, and no significant reflex tachycardia was observed. However, a 0.3-mg. increment increase in bolus dose (0.6 mg./kg.) produced a 40-mm. Hg decrease in mean systolic pressure and a significant compensatory reflex tachycardia (Table I).

Over a bolus dose range of 0.6–1.8 mg./kg., an essentially linear reduction in mean systolic and diastolic blood pressure was observed; with each 0.3-mg. increment in dose up to a total of 1.8 mg./kg., mean systolic blood pressure was reduced about 14 mm. Hg (Table I and Fig. 1).

The vasodepressor response to a bolus injection of β-hydroxylamine cinnamonyl hydroxamic acid at all dose levels was prompt and short in duration; and in no experiment did the duration of response exceed 1 min. A slightly increased respiratory rate (4–6/min.) was usually noted when systolic blood pressure was reduced sufficiently (40 mm. Hg) to recruit reflex tachycardia.

Repeated intravenous bolus injections of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg.) at 5- and 2-min. intervals over a period of 26 min. showed no evidence of tachyphylaxis or anaphylaxis; *i.e.*, the vasodepressor response neither decreased nor increased (Fig. 2).

In 30 dogs, a continuous intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid at the rate of 0.3 mg./kg./min. produced a sustained decrease in average blood pressure of 14 ± 2.3 mm. Hg. Doubling the dose (0.6 mg./kg./min.) decreased average blood pressure 33 ± 6.2 mm. Hg. Sustained infusions of β-hydroxylamine cinnamonyl hydroxamic acid at the latter dose level for periods up to 2 hr. showed no cumulative effects. Qualitative analysis for methemoglobin (6) in blood samples taken at 10-min. intervals over a 2-hr. period of infusion of β-hydroxylamine cin-

[1] Confirmational elemental and structural analyses were carried out by Alfred Bernhardt, Max Planck Institute, West Germany.

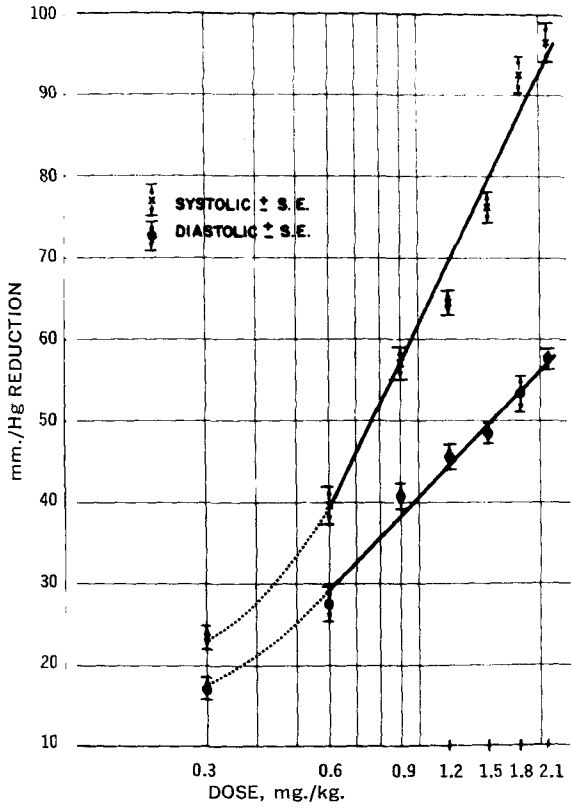


Figure 1—*Log dose–response plot of systolic and diastolic pressure reductions produced by progressively increasing incremental bolus doses of β-hydroxylamine cinnamonyl hydroxamic acid. Mean ± SD of response at each dose level was determined from 6–11 separate responses in an equal number of dogs. A least-squares fit applied to the dose range of 0.6–2.1 mg./kg. was employed to show that the response to the minimal effective dose was greater than that to any subsequent incremental increase.*

namonyl hydroxamic acid at several dose levels (0.6, 1.2, and 1.8 mg./kg./min.) was negative. Regardless of the dose–response relationship, blood pressure always returned promptly to preinfusion control levels on discontinuing β-hydroxylamine cinnamonyl hydroxamic acid. This is illustrated by a typical experiment in Fig. 3.

In 11 dogs rendered hypotensive (−30 ± 2.5 mm. Hg) by means of the standard infusion of β-hydroxylamine cinnamonyl hydroxamic acid, epinephrine HCl injected intravenously (1.5 mcg./kg.) produced a normal vasopressor response in all subjects. This response clearly suggested that α-adrenergic receptors were unaffected by the β-hydroxylamine cinnamonyl hydroxamic acid-induced vasodilatation.

Response to β-Hydroxylamine Cinnamonyl Hydroxamic Acid in the Presence of an Antihistamine—To determine if the vasodepressor response to β-hydroxylamine cinnamonyl hydroxamic acid was due in part to the release of endogenous histamine, β-hydroxylamine cinnamonyl hydroxamic acid was administered 30 min. before and after producing histamine block in seven dogs. An intravenous test dose of histamine diphosphate (0.01 mg./kg.) was injected 15 min. before and after the intravenous administration of diphenhydramine HCl (2.0 mg./kg.) in order to examine the efficacy of the histamine block. After establishing a satisfactory but varying degree of histamine block in each subject (Fig. 4), the intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg./min.) produced a vasodepressor response in all subjects that was identical in pattern and degree to the preblock control response (Table II).

Response to β-Hydroxylamine Cinnamonyl Hydroxamic Acid in the Presence of Atropine—To determine if β-hydroxylamine cinnamonyl hydroxamic acid produced elaboration of mediator acetylcholine, six dogs were treated with atropine sulfate (0.5 mg./kg. i.v.) 30 min. after a standard control infusion of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg./min.). An intravenous test dose of acetylcholine Cl (5.0 mcg./kg.) was administered 10 min. before and 40 min. after the atropine block. The marked vaso-

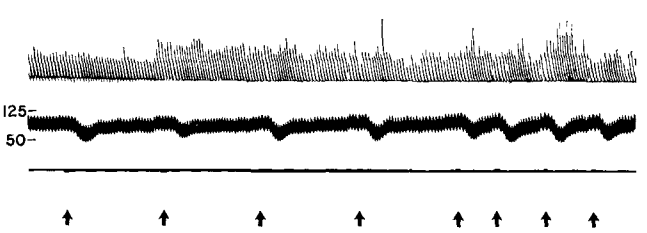


Figure 2—*Montage (eight frames) of a typical experiment showing complete absence of tachyphylaxis and anaphylaxis to repeated intravenous bolus injections of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg.) in the anesthetized dog. β-Hydroxylamine cinnamonyl hydroxamic acid was injected four times at 5-min. intervals and four times at 2-min. intervals as indicated. Regardless of the interval, each injection of β-hydroxylamine cinnamonyl hydroxamic acid produced a consistent 30-mm. reduction in average blood pressure. Respiratory rate (11/min.) and duration of response (50 sec.) to β-hydroxylamine cinnamonyl hydroxamic acid were unaffected by the repeated close-order injections.*

depressor and respiratory responses to injected acetylcholine were adequately blocked by atropine in all experiments (Fig. 5), and the postatropine vasodepressor response to the standard infusion of β-hydroxylamine cinnamonyl hydroxamic acid was unaltered (Table II). Because atropine adequately blocked the unusually dramatic vasodepressor response to the second challenging test dose of acetylcholine, it was clear that the lesser, yet characteristic, degree of vasodepression produced by the standard β-hydroxylamine cinnamonyl hydroxamic acid infusion was not mediated by acetylcholine.

Response to β-Hydroxylamine Cinnamonyl Hydroxamic Acid in the Presence of Physostigmine—To determine if the vasodepressor action of β-hydroxylamine cinnamonyl hydroxamic acid was potentiated or prolonged by acetylcholinesterase inhibition, the standard infusion of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg./min.) was administered before and after the intravenous administration of physostigmine salicylate (0.1 mg./kg.). In four of seven dogs, this acetylcholinesterase blocking dose of physostigmine produced measurable skeletal muscle fasciculations, with moderate increases in average blood pressure (Table II). Both the muscle fasciculation and elevated blood pressure persisted until β-hydroxylamine cinnamonyl hydroxamic acid was infused; however, the pattern and degree of vasodepressor response to β-hydroxylamine cinnamonyl hydroxamic acid were unaltered (Table II). The somatic neuroeffector response to physostigmine was regarded

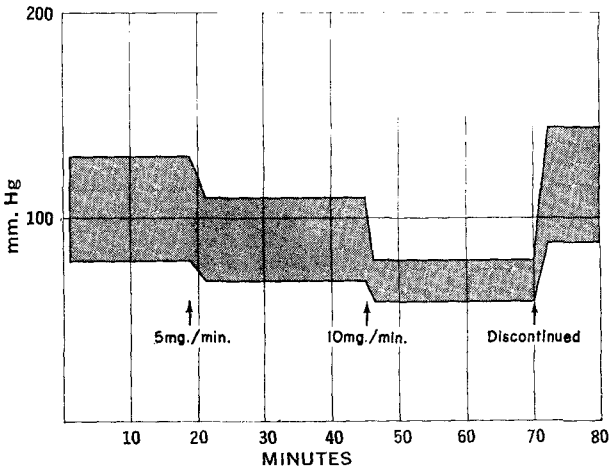


Figure 3—*Typical pattern of vasodepressor response to a continuous intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid at two dose levels. Note particularly the stability of the staircase reduction in systolic blood pressure at each dose level and the rapid recovery—with slight overshoot—of systolic-diastolic and pulse pressures following abrupt cessation of the β-hydroxylamine cinnamonyl hydroxamic acid infusion. Infusion rates at 5 and 10 mg./min. equal 0.3 and 0.6 mg./kg./min., respectively.*

Table II—Effect of Pretreatment with a Number of Blocking Agents on the Vasodepressor Action of β-Hydroxylamine Cinnamonyl Hydroxamic Acid in the Anesthetized Dog

1 Blocking Agent[a], Intravenous Dose	2 Average Control, Blood Pressure ± *SE*, mm. Hg	3 Response to Agent, Blood Pressure ± *SE*	4 Control Response to β-Hydroxylamine Cinnamonyl Hydroxamic Acid, Blood Pressure ± *SE*	5 Response to β-Hydroxylamine Cinnamonyl Hydroxamic Acid after Agent	6 Significance, Column 4 *versus* 5
Diphenhydramine, 2.0 mg./kg. (N = 7)	112 ± 7.7	−40 ± 6.8	−35 ± 2.1	−34 ± 2.4	N.S.
Atropine, 0.5 mg./kg. (N = 6)	111 ± 7.6	−1 ± 1.6	−27 ± 2.7	−26 ± 2.4	N.S.
Physostigmine, 0.1 mg./kg. (N = 7)	108 ± 7.5	+16 ± 7.1	−26 ± 2.4	−30 ± 2.5	N.S.
Dichloroisoproterenol, 10 mg./kg. (N = 5)	106 ± 5.5	−43 ± 2.1	−30 ± 1.7	−29 ± 1.6	N.S.
Chlorpromazine, 1.5 mg./kg. (N = 5)	110 ± 8.5	−5 ± 2.4	−33 ± 4.3	−32 ± 3.0	N.S.
Phenoxybenzamine, 12 mg./kg. (N = 6)	106 ± 7.7	−25 ± 1.3	−30 ± 1.5	−29 ± 3.0	N.S.

[a] A minimum of 30 min. was permitted to elapse between the administration of the blocking agent and the start of the standard infusion of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg./min.). The average control blood pressure in Column 2 was computed by adding the sums of the systolic and diastolic pressures and dividing by 2. The immediate response to the blocking agent (Column 3) was tabulated as the deviation from the average blood pressure in Column 2. The values in Column 4 represent the response to a standard β-hydroxylamine cinnamonyl hydroxamic acid infusion before the administration of the blocking agent. The significance in Column 6 was determined by the Student t test in a self-paired analysis.

as evidence of the significant acetylcholinesterase blocking action of physostigmine.

Atropine block (0.5 mg./kg.) of the muscarinic actions of elevated acetylcholine titers induced by physostigmine inhibition of acetylcholinesterase also failed to modify the characteristic vasodepressor response to β-hydroxylamine cinnamonyl hydroxamic acid. These experiments were identical in protocol and pattern of response to β-hydroxylamine cinnamonyl hydroxamic acid to those illustrated in Fig. 5.

Response to β-Hydroxylamine Cinnamonyl Hydroxamic Acid in the Presence of β-Adrenergic Blockade—To determine if β-hydroxylamine cinnamonyl hydroxamic acid produced its vasodepressor action through direct stimulation of β-adrenergic receptors, five dogs were treated with dichloroisoproterenol (10 mg./kg. i.v.) after a control standard infusion of β-hydroxylamine cinnamonyl hydroxamic acid. A challenging dose of isoproterenol (0.3 mg./kg. i.v.) was administered 30 min. after dichloroisoproterenol to appraise the adequacy of the β-adrenergic block in all subjects; the standard intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid produced its characteristic vasodepressor response (Table II).

Actions of β-Hydroxylamine Cinnamonyl Hydroxamic Acid in the Presence of α-Adrenergic Blockade—In six dogs pretreated with phenoxybenzamine HCl (12.0 mg./kg.) and five dogs pretreated with chlorpromazine HCl (1.5 mg./kg.), following epinephrine (1.5 mcg./kg.) verification of α-receptor blockade, the standard infusion of β-hydroxylamine cinnamonyl hydroxamic acid produced a vasodepressor response that was similar in all respects to the pretreated control response in 11 subjects (Table II).

Because β-hydroxylamine cinnamonyl hydroxamic acid failed to show β-adrenergic stimulating activity, it became of interest to determine if the epinephrine reversal produced by α-adrenergic blockade would be exaggerated or otherwise attenuated by β-hydroxylamine cinnamonyl hydroxamic acid. In the 11 α-block experiments previously described, wherein epinephrine reversal was observed in all subjects, a second bolus dose of epinephrine (1.5 mcg./kg.) administered during the infusion of β-hydroxylamine cinnamonyl hydroxamic acid now produced a clear vasopressor response (Fig. 6). On discontinuing the infusion of β-hydroxylamine cinnamonyl hydroxamic acid and again repeating the epinephrine, the classical epinephrine reversal was once again observed.

The β-hydroxylamine cinnamonyl hydroxamic acid-induced restoration of the epinephrine vasopressor response in the α-blocked subject (Arrow 2, Fig. 6) was in marked contradistinction to the epinephrine reversal observed before and after the infusion of β-hydroxylamine cinnamonyl hydroxamic acid (Arrows 1 and 3, Fig. 6). This response, produced by β-hydroxylamine cinnamonyl hydroxamic acid, could not be attributed to a temporary chronotropic action of epinephrine since a reflex bradycardia was observed in the majority of experiments.

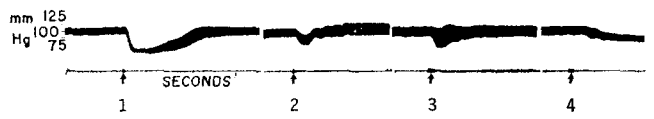


Figure 4—*Influence of histamine block on the vasodepressor actions of β-hydroxylamine cinnamonyl hydroxamic acid in the anesthetized dog. Histamine diphosphate (0.01 mg./kg.) was injected before and after (Arrows 1 and 3) the intravenous administration of a blocking dose (2.0 mg./kg.) of diphenhydramine HCl (Arrow 2). The initial histamine injection reduced blood pressure from 115/85 to 35/20 mm. Hg, while a second challenging dose of histamine, delivered 15 min. after diphenhydramine, reduced blood pressure from 120/85 to 110/40 mm. Hg. The confirmed and satisfactory degree of histamine block failed to modify the characteristic pattern and degree of vasomotor depression produced by a standard intravenous infusion (Arrow 4) of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg./min.). Evaluation of the systolic–diastolic and pulse pressure morphologies exemplified by histamine, diphenhydramine, and β-hydroxylamine cinnamonyl hydroxamic acid showed that endogenous histamine liberation is inconsequential to the consistent and stable blood pressure reduction produced by β-hydroxylamine cinnamonyl hydroxamic acid.*

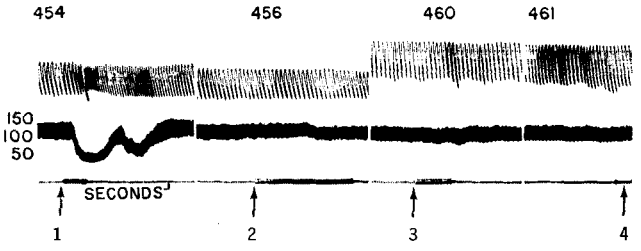


Figure 5—*Influence of cholinergic block on the vasodepressor actions of β-hydroxylamine cinnamonyl hydroxamic acid in the anesthetized dog. The vasomotor and respiratory responses to an intravenous test dose of acetylcholine Cl (5.0 mcg./kg.) were determined before (Arrow 1) and after (Arrow 3) the administration of 0.5 mg./kg. atropine sulfate (Arrow 2) in order to ascertain the adequacy of the atropine block. The atropine block, confirmed by the absence of significant respiratory and vasomotor responses to a challenging dose of acetylcholine (Arrow 3), did not alter the characteristic pattern of vasomotor responses to a standard infusion (0.6 mg./kg./min.) of β-hydroxylamine cinnamonyl hydroxamic acid (Arrow 4). It is apparent in this typical experiment that the vasodepressor action of β-hydroxylamine cinnamonyl hydroxamic acid is not dependent upon cholinergic mediators. (Respiration, upper tracing.)*

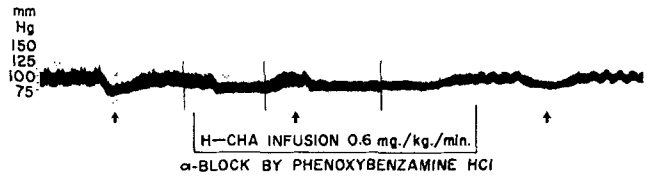


Figure 6—*Montage of a typical experiment demonstrating negation of α-adrenergic blockage by β-hydroxylamine cinnamonyl hydroxamic acid. In the phenoxybenzamine-pretreated dog (12.0 mg./kg.), a bolus injection of epinephrine (1.5 mcg./kg.) produced marked vasodepression (Arrow 1) and confirmed the adequacy of the α-block. Repeating the epinephrine during the infusion of β-hydroxylamine cinnamonyl hydroxamic acid now produced a definite vasopressor response (Arrow 2). Five minutes after discontinuing the infusion of β-hydroxylamine cinnamonyl hydroxamic acid, a third injecton of epinephrine (Arrow 3) showed reestablishment of the phenoxybenzamine-induced α-blockade. The infusion of β-hydroxylamine cinnamonyl hydroxamic acid clearly lifted the confirmed α-block; however, it did not permanently displace phenoxybenzamine nor prevent rapid reestablishment of the α-block.*

In control experiments utilizing direct-acting vasodilator agents (nitroglycerin, sodium nitrite, and papaverine) and hemorrhage (12% of calculated blood volume) to reduce systemic blood pressure about 30 mm. Hg, an intravenous bolus injection of epinephrine during the induced hypotension of the α-blocked dog failed to produce a vasopressor response. Only in the hemorrhaged hypotensive subject did epinephrine produce a small chronotropic response which was inadequate to modify significantly the epinephrine reversal produced by phenoxybenzamine α-blockade.

DISCUSSION

The most significant observations in this study relate to the ability of β-hydroxylamine cinnamonyl hydroxamic acid to exert a consistent hypotensive action on sustained intravenous infusion and to restore the pressor action of epinephrine in an established α-adrenergic blockade.

The vasodepressor responses to bolus injections of β-hydroxylamine cinnamonyl hydroxamic acid over a wide dose range were of short duration, lasting 50 sec. on the average, and no tachyphylaxis or anaphylaxis was observed following repeated close-order intravenous administration (Fig. 2). Prolonged intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid, producing 30-mm. Hg reductions of average systemic blood pressure for periods up to 4 hr., produced no apparent cumulative effects.

The ultrashort duration of action of β-hydroxylamine cinnamonyl hydroxamic acid is apparently related to its unique molecular structure. β-Aminopropionohydroxamic acids, synthesized and studied recently by Coutts *et al.* (7), produced hypotension in the anesthetized cat lasting from 5 to 120 min. following a single intravenous bolus injection. Substituting cyclic tertiary amines on the β-carbon of propionohydroxamic acids—in contrast to arylhydroxylamine substitutions—probably accounts for the minimal fivefold increase in duration of action.

The lowering of systemic blood pressure by β-hydroxylamine cinnamonyl hydroxamic acid in the anesthetized dog was not modified significantly by any of the mediator blocking agents studied. The pharmacodynamic action of β-hydroxylamine cinnamonyl hydroxamic acid, therefore, appears to be direct on vascular smooth muscle.

Pretreatment of dogs with large doses of diphenhydramine (2.0 mg./kg.) and atropine sulfate (0.5 mg./kg.) consistently failed to produce any alteration in the blood pressure responses to intravenous bolus injections or sustained infusions of β-hydroxylamine cinnamonyl hydroxamic acid. It was clear in all experiments that β-hydroxylamine cinnamonyl hydroxamic acid was not acting directly on histaminergic or cholinergic (muscarinic) receptors (Figs. 4 and 5).

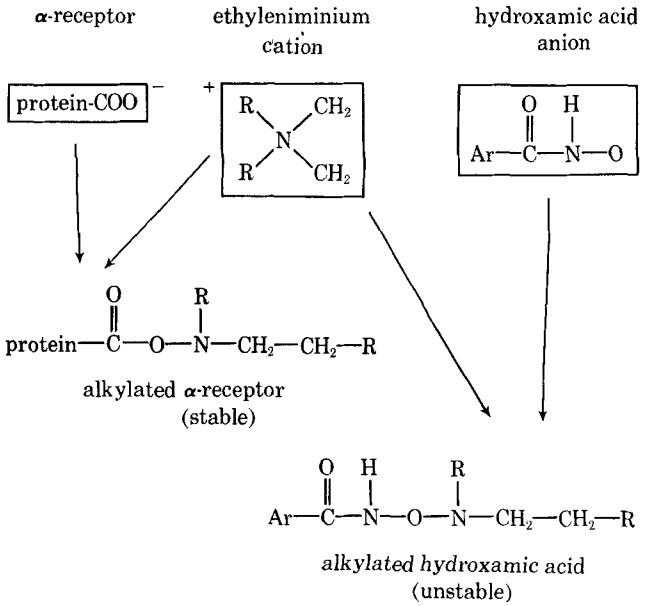
The infusion of β-hydroxylamine cinnamonyl hydroxamic acid in the physostigmine-pretreated dog produced no greater vasodepressor response than that observed in control experiments. Skeletal muscle fasciculations observed in four of seven experiments following intravenous administration of physostigmine was promptly and completely abolished by the standard intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid (0.6 mg./kg./min.). This observation strongly suggested a direct action of β-hydroxylamine cinnamonyl hydroxamic acid on skeletal muscle contractile mechanisms and provided further evidence that the action of β-hydroxylamine cinnamonyl hydroxamic acid is independent of cholinergic mediation. This antinicotinic action of β-hydroxylamine cinnamonyl hydroxamic acid is being investigated further.

The use of a β-receptor blocking agent (dichloroisoproterenol) demonstrated that β-hydroxylamine cinnamonyl hydroxamic acid did not produce its vasodepressor action through stimulation of β-receptor mechanisms. A bolus injection or a sustained infusion of β-hydroxylamine cinnamonyl hydroxamic acid in the β-blocked animal lowered systemic blood pressure to the same degree as in untreated controls (Table II).

α-Receptor blocking agents (phenoxybenzamine and chlorpromazine) in no way modified the vasodepressor response to β-hydroxylamine cinnamonyl hydroxamic acid. On the other hand, an infusion of β-hydroxylamine cinnamonyl hydroxamic acid was clearly able to restore—for the duration of the infusion—a confirmed α-blockade produced by either blocking agent; *i.e.*, during an infusion of β-hydroxylamine cinnamonyl hydroxamic acid, an intravenous injection of epinephrine that previously produced marked vasodilatation now produced a pronounced vasoconstriction accompanied by reflex bradycardia (Fig. 6). It is interesting indeed that the restoration of the epinephrine vasopressor response in the α-blocked dog produced by β-hydroxylamine cinnamonyl hydroxamic acid in no way interfered with normal cardiovascular buffer reflexes.

The apparent α-block lift produced by β-hydroxylamine cinnamonyl hydroxamic acid was both peculiar and unique inasmuch as the several types of α-block lift previously reported (8, 9) are seemingly of a different nature. For example, permanent α-block lift may be produced by trypsin enzymatic hydrolysis of haloalkylamine-alkylated α-receptors, and temporary lift may be produced by large doses of ephedrine or methoxamine. β-Hydroxylamine cinnamonyl hydroxamic acid-induced α-block lift occurred only for the duration of a standard intravenous infusion, and the α-receptor block produced by the haloalkylamines employed (phenoxybenzamine and chlorpromazine) was reestablished immediately after discontinuing the infusion (Fig. 6).

It now appears well established that β-haloalkylamines ionize to form ethyleniminium cations which alkylate and covalently bond to the carboxyl sites of α-adrenergic receptor enzyme profiles (10). In Scheme I, it is postulated that hydroxylamine cinnamonyl hydroxamic acid, a highly polar aralkyl molecule with anionic properties, would have a strong physicochemical affinity for the ethyleniminium cation. Acting as an anion, hydroxylamine cinnamonyl hydroxamic acid may combine with the labile cationic fraction



Scheme I

(ethyleniminium) of the β-haloalkylamine and prevent subsequent alkylation of "reserve adrenergic receptors" (11) brought forth through the vasodilator action of hydroxylamine cinnamonyl hydroxamic acid. In this way, hydroxylamine cinnamonyl hydroxamic acid would both restore the epinephrine vasopressor response and protect spare α-receptors from alkylation.

The β-hydroxylamine cinnamonyl hydroxamic acid–ethyleniminium bonding (Scheme I) is clearly unstable since it can be maintained only by a continuous infusion of β-hydroxylamine cinnamonyl hydroxamic acid. The dual actions of β-hydroxylamine cinnamonyl hydroxamic acid—rendering spare receptors accessible and bonding ethyleniminium—only mimic an α-block lift inasmuch as the alkylated α-receptors are not inactivated. This was evident by the observation that epinephrine reversal was immediately reinstituted on discontinuing the infusion of β-hydroxylamine cinnamonyl hydroxamic acid.

The maintenance of vascular smooth muscle tone is related to controlled neural and adrenal baroreceptor-triggered adrenomedullary release of catecholamines (12). Recent evidence indicates that norepinephrine may even be synthesized locally within the arterial wall (13), and the interaction of epinephrine and/or norepinephrine and adenosine triphosphate provides necessary energy for smooth muscle contraction and maintenance of vascular tone (14).

Adrenomedullary release of catecholamines has been shown to be adenosine triphosphate dependent, and it can be blocked with *N*-ethylmalimide which interferes with adenosine triphosphotase (15). The direct inhibitory action of β-hydroxylamine cinnamonyl hydroxamic acid at the vascular smooth muscle level is perhaps like that of *N*-ethylmalimide at the adrenomedullary level; *i.e.*, β-hydroxylamine cinnamonyl hydroxamic acid most likely interferes with energy-dependent processes within the arterial smooth muscle wall. The vasodilatation induced by β-hydroxylamine cinnamonyl hydroxamic acid may also be due in part to the formation of organometallic complexes (β-hydroxylamine cinnamonyl hydroxamic acid–Ca or β-hydroxylamine cinnamonyl hydroxamic acid–Mg) with calcium or magnesium ions that would interfere with adenosine triphosphate-dependent energy-generating mechanisms (15).

The metabolic fate of β-hydroxylamine cinnamonyl hydroxamic acid is unknown at this time. Utilizing the ferric chloride ($FeCl_3$) method for determining the hydroxamic acid moiety in β-hydroxylamine cinnamonyl hydroxamic acid, all attempts to detect β-hydroxylamine cinnamonyl hydroxamic acid in serum, whole blood, and ureteral urine met with only limited success, and then only after a 4-hr. period of administering the standard intravenous infusion of β-hydroxylamine cinnamonyl hydroxamic acid. In a 20.0-kg. dog, this constituted a total dose of 2.88 g. The rapid inactivation of β-hydroxylamine cinnamonyl hydroxamic acid in body fluids is in complete accord with its ultrashort duration of action.

Chemical analyses for methemoglobin in blood samples taken at 30-min. intervals over a 4-hr. period of constant infusion (0.6 mg./kg./min.) of β-hydroxylamine cinnamonyl hydroxamic acid showed only a trace toward the end of the infusion period.

Preliminary studies of the toxicity of β-hydroxylamine cinnamonyl hydroxamic acid indicate that the intraperitoneal LD_{50} in the rat is of the order of 350 mg./kg. Lethal doses of β-hydroxylamine cinnamonyl hydroxamic acid, administered intraperitoneally, produced CNS depression, hypoxemia, and marked cyanosis, followed by clonic convulsions and death.

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