Muscle Relaxant Action of β-Hydroxylamine Cinnamonyl Hydroxamic Acid in Isolated Organs

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Abstract □ β-Hydroxylamine cinnamonyl hydroxamic acid produced muscle relaxation in the guinea pig trachea, the rabbit jejunum, and the rabbit atria. This relaxing action was not blocked by α- or β-blocking agents, atropine and diphenhydramine; however, it could be antagonized with epinephrine, acetylcholine, and histamine. The degree of muscle relaxation was dose dependent and expressed itself in decreased force exerted and decreased frequency of spontaneous contractions. The β-hydroxylamine cinnamonyl hydroxamic acid-induced muscle relaxation could not be explained in terms of any known neurohumoral mediator inhibition and appears to be direct and attributable to the unique molecular structure of the hydroxamic acid itself.

Keyphrases □ β-Hydroxylamine cinnamonyl hydroxamic acid—muscle relaxant activity *in situ* □ Muscle relaxant activity, *in situ*—hydroxylamine cinnamonyl hydroxamic acid □ Hydroxamic acid, hydroxylamine cinnamonyl—muscle relaxant activity *in situ*

β-Hydroxylamine cinnamonyl hydroxamic acid (I) has been studied as an example of the generic group of hydroxamic acids. Earlier it was reported (1) that this compound showed vasomotor properties and seems to be active as a muscle-relaxing agent. The cardiovascular properties established the hypotensive action of this hydroxamic acid.

During these studies it also was observed that I was able to antagonize physostigmine-induced muscle fasciculations of skeletal muscles. Previously, it had been concluded that the hypotensive and cardiovascular actions of I were a result of interference of energy-generating mechanisms and that this property was inherent in the molecular structure of this hydroxamic acid (1).

To examine the muscle-relaxant properties of this compound in more detail and to lend support for its direct action, it was studied in three isolated organ systems. The three organ systems are representative of the adrenergic, cholinergic, and histaminergic systems.

To examine the effect on the adrenergic system, the isolated rabbit atria were selected; this tissue also exemplifies a muscle system with a strong autogenous contractile drive. The rabbit jejunum served as the model of the cholinergic system, a muscle contractile system with a moderate autogenous drive. The guinea pig trachea served as the representative histaminergic system and as a muscle system with a weak autogenous contractile drive.

EXPERIMENTAL

Adrenergic System—Twenty-three mongrel rabbits of either sex, weighing 2–3 kg., were used. Each animal was stunned with a blow on the back of the neck and the heart was excised immediately. The organ was quickly washed with warm oxygenated Locke solution to remove any residual blood. While the heart remained submerged in Locke solution, the atria were separated from the ventricles. The spontaneously beating atria were suspended in warm (37.5°) oxygenated Locke solution in a bath of 50 ml. Contractions were measured with a force-displacement transducer[1] and recorded with a polygraph[2]. The instrument was calibrated so that a 2-cm. deflection was equal to 1 g. tension force. The organ was permitted to attain equilibrium in the organ bath for 20–40 min., with frequent washings, before any experimental or control readings were taken.

After equilibrium, the number of beats per minute (frequency) was obtained in the following manner: the number of beats was counted for every alternate 6 sec. in a 2-min. period and averaged as the mean number of beats per minute. This was repeated at least three times for each experimental or control event taken. The respective means so obtained were then averaged as the mean of the means. The Student *t* test with self-paired analysis was applied for statistical evaluation.

Cholinergic System—Twenty-one mongrel rabbits of either sex were sacrificed in the same manner as already described. A midline incision was made, and approximately 5 cm. of jejunum was excised 20–25 cm. distal to the pylorus. The gut lumen was gently washed with warm (37.5°) Tyrode solution to remove all contents. Approximately 2.5 cm. of jejunum was suspended in warm Tyrode solution saturated with 95% oxygen and 5% carbon dioxide in an organ bath of the dimensions already described. Contractions were measured by the same force transducer and polygraph. The calibration was that a 2-cm. deflection equaled 1 g. force. The frequency of contractions was counted manually and evaluated in the same manner as described for the atria. The jejunum was equilibrated for 30 min. in the organ bath, with frequent washings, before any experimental or control readings were taken.

Histaminergic System—Twenty-four Hartley short hair guinea pigs of either sex were sacrificed in the similar manner as the rabbits. The ventral side of the neck was opened and about 3 cm. of trachea was removed. The trachea was washed and cleaned. Spiral tracheal strips of 0.5-cm. width and 2.0-cm. length (2) were prepared and mounted in the organ bath containing warm Tyrode solution saturated with 95% oxygen and 5% carbon dioxide. The organ bath used was the same type as already described. The trachea was permitted a 15–30-min. equilibrium period, with frequent washings, before any experimental or control readings were taken. Gram-force was recorded as described for the rabbit jejunum. No frequency measurements were taken.

All drugs used were diluted in either Locke or Tyrode solution and given in the least volume possible to produce minimal osmotic shock to the tissues. The hydroxamic acid was dissolved in either Tyrode or Locke solution without altering the pH of these solutions. The concentration of the β-hydroxylamine cinnamonyl hydroxamic acid was 50 mg./ml. for the atria, 10 mg./ml. for the jejunum, and 2.5 mg./ml. for the trachea, which, in turn, made the bath concentrations of 1 mg./ml. for the atria, 0.2 mg./ml. for the jejunum, and 0.05 mg./ml. for the trachea possible.

Calcium determinations of the organ bath fluid were carried out by the method of Sherrick and de la Huerga (3).

RESULTS

Effects of β-Hydroxylamine Cinnamonyl Hydroxamic Acid on Spontaneously Beating Isolated Rabbit Atria—Alterations of the contractile rate of the spontaneously beating rabbit atria were observed only after relatively high doses of I (0.8–1.0 mg./ml. bath). Changes in the contractile force become evident at 0.4 mg./ml. bath; significant changes were observed at concentrations of 1 mg./ml. bath (Figs. 1 and 2). Incremental increases in concentrations of I from 0.8 to 2.0 mg./ml. bath produced a log dose linear reduction for both frequency and gram-force exerted by the atria (Figs. 1 and 2).

[1] FT03C, Grass.
[2] Grass model 15.

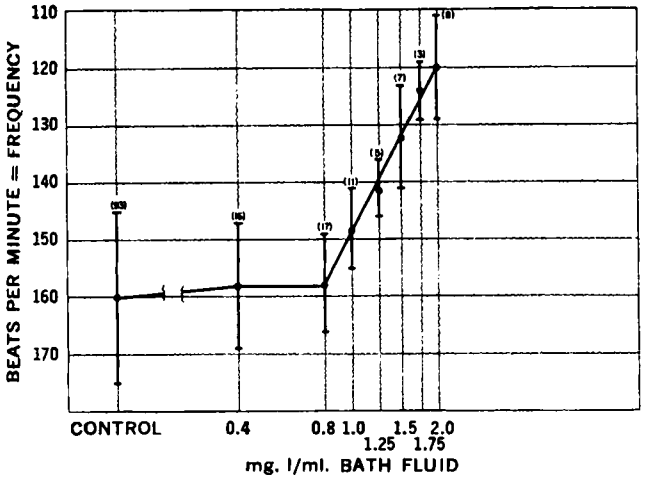

Figure 1—*Log dose-response plot of beats per minute (frequency) of the isolated rabbit atria. A log linear response was observed with incremental increases of concentrations of I when a least-squares fit was applied for the range of 0.8–2.0 mg./ml. bath. Frequencies were recorded as the mean of the means with the standard deviations indicated. The number of means used for each concentration is indicated by the number in parentheses.*

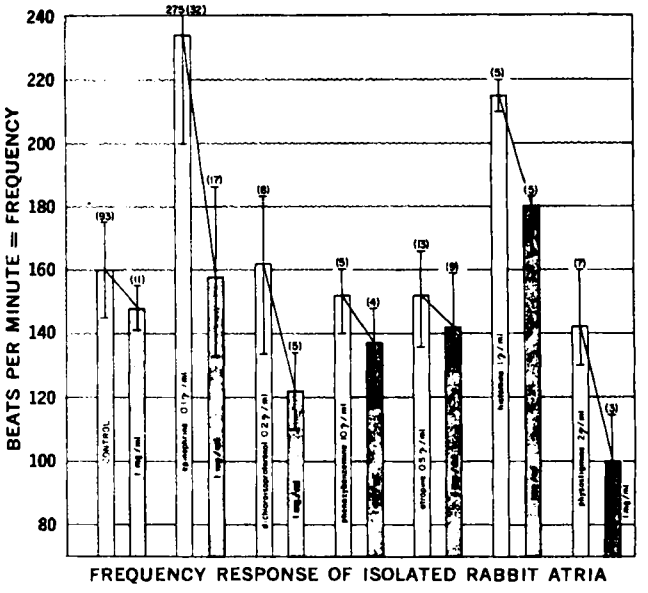

Figure 3—*Histogram of beats per minute (frequency) of the isolated rabbit atria treated with I in the presence of various agonists and blocking agents. The shaded bars represent the addition of 1.0 mg. I/ml. bath. Frequencies were recorded as the mean of the means with the standard error indicated. The number of means evaluated is indicated by the number in parentheses. The concentrations for the agonists and blocking agents were expressed as per milliliter of bath. In the histogram the effect of the agonist after the block was omitted since no significant change of frequency was recorded.*

Higher concentrations of I produced a state of contracture of the atria associated with an increase in tension and a further reduction of frequency with no alteration of amplitude.

To determine the efficacy of I on the force and rate of the atrial contractions, the agonists epinephrine and histamine were selected. Under control conditions a standard dose of I (1 mg./ml. bath) produced an average reduction of the frequency of the contractions by 12 beats/min., while a simultaneous reduction of an average of 0.48 gram-force occurred. After stimulating the atria with 0.1 mcg. epinephrine/ml. bath, the addition of the standard dose produced an average reduction of 76 beats/min. while at the same time a 0.79-g. average reduction of force occurred. Stimulating the atria with 1 mcg. histamine/ml. bath followed with the standard dose of I led to an average reduction of 35 beats/min. and an average reduction of 1.40 gram-force (Figs. 3 and 4).

To ascertain if the actions of I were direct and not through release or inhibition of mediators, the effects of several blocking agents on the response of I were studied. In all cases the efficacy of the block was tested by challenging the tissue with the appropriate agonist. After the addition of 0.2 mcg. dichloroisoproterenol/ml. bath, no changes in frequency and gram-force from the control values were observed. The addition of the standard dose of I in the presence of the challenger epinephrine and the β-blocking agent in the tissue bath now produced an average reduction of frequency of 40 beats/min. and an average reduction of gram-force of 0.53 g. The addition of 10 mcg./ml. phenoxybenzamine to the organ bath resulted in a moderate reduction in frequency of the atria with no change in gram-force. After verifying the adequacy of the block with norepinephrine, the standard dose of I produced, in the presence of the block and the agonist, an average reduction of 15 beats/min. and a 0.22-

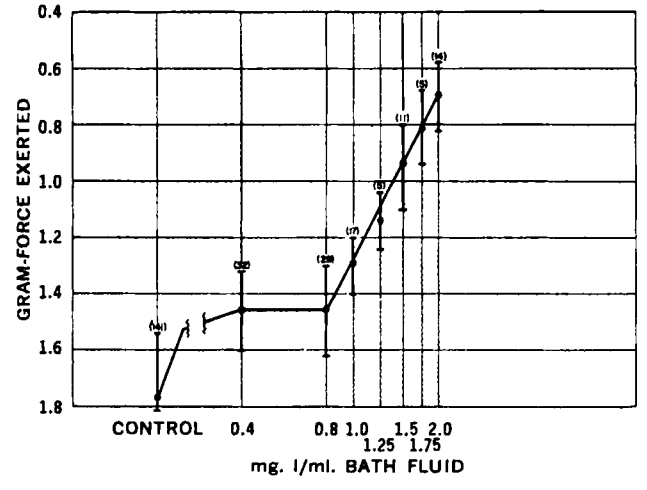

Figure 2—*Log dose-response plot of the gram-force exerted by the isolated rabbit atria after incremental concentration increases of I. A log linear response was obtained when a least-squares fit was applied for the concentration range of 0.8–2.0 mg./ml. bath. Gram-force was recorded as the mean of the means with the standard deviations indicated. The number of means used for each incremental increase is indicated by the number in parentheses.*

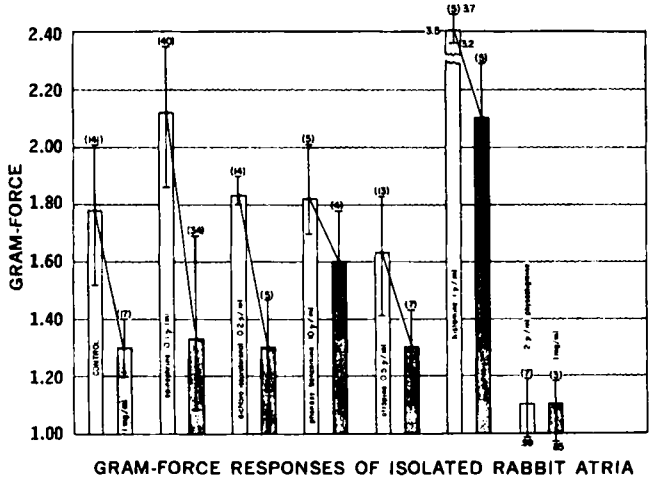

Figure 4—*Histogram of the reduction of gram-force exerted by the isolated rabbit atria. The shaded bars represent I (1.0 mg./ml. bath). The gram-force was tabulated as the mean of the means with the standard deviation indicated for each bar. The number in parentheses represents the means evaluated for this bar. The responses of the atrium are shown after agonists and in the presence of blocking agents. The effect of agonists after blocking agents was omitted since no significant change in response was calculated.*

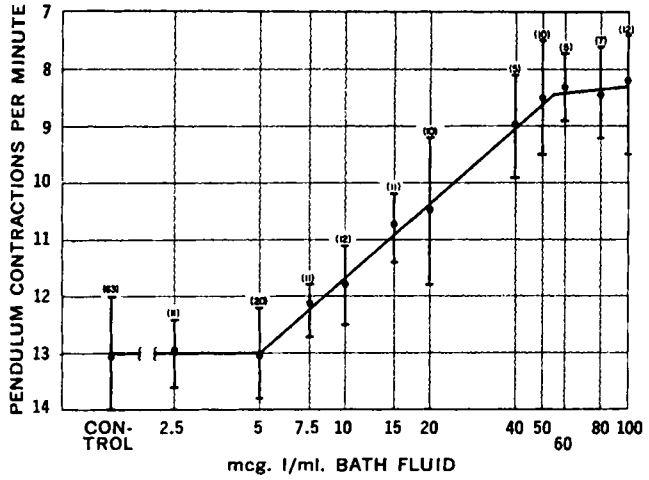

Figure 5—*Log-dose response plot of pendulum contractions (frequency) of the isolated rabbit jejunum. A log linear response was observed with incremental increases of concentrations of I when a least-squares fit was applied for the concentration range of 5.0–50.0 mcg./ml. bath. Before 5 mcg./ml. bath of the compound, a level response was noted; at concentrations greater than 50.0 mcg./ml., a ceiling effect was observed. Frequencies were recorded as the mean of the means with the standard deviations indicated. The number of means used for this concentration is indicated by the number in parentheses.*

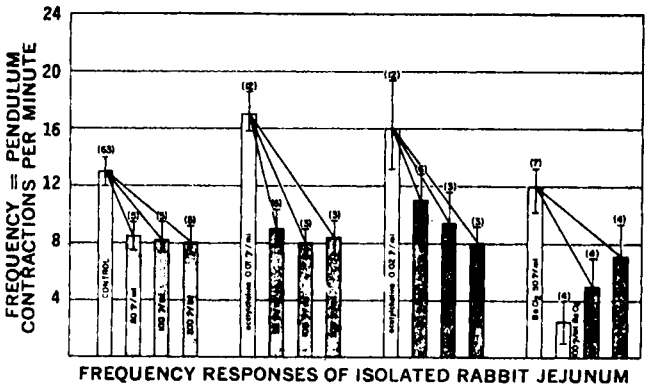

Figure 7—*Histogram of the pendulum contractions per minute (frequency) of the isolated rabbit jejunum treated with I in the presence of two agonists and two different concentrations for one agonist. The shaded bars represent I at 50, 100, and 200 mcg./ml. as indicated. All concentrations are per milliliter of bath. The frequencies were recorded as the mean of the means for each bar with the standard deviations shown. The number of means used for each bar is indicated by the number in parentheses.*

gram-force reduction. The addition of 0.5 mcg./ml. of atropine to the bath produced a moderate reduction in both the frequency and gram-force of the atria in comparison to the control values. After ascertaining the adequacy of the atropine block with 0.01 mcg. acetylcholine/ml. bath, the standard dose of I produced an average reduction of 10 beats/min. in frequency and a 0.33-gram-force reduction from the atropine values. Physostigmine (2 mcg./ml. bath) produced a marked reduction of the frequency and gram-force exerted by the atria. The addition of the standard dose of I to the bath produced a further reduction of the frequency of 42 beats/min., while no change was observed in the gram-force (Figs. 3 and 4).

Effects of β-Hydroxylamine Cinnamonyl Hydroxamic Acid on Isolated Rabbit Jejunum—Minimum concentrations of I to inhibit spontaneously occurring rhythmic pendulum contraction and the gram-force exerted by the jejunum were 7.5 mcg./ml. Incremental increases of I from 5 to 50 mcg./ml. yielded a linear log dose reduction in frequency of the spontaneous contractions. At concentrations above 50 mcg./ml., a ceiling effect was observed (Fig. 5). Incremental increases of I in the range of 5–20 mcg./ml. produced a linear log dose reduction in the gram-force exerted by the jejunum. At concentrations above 20 mcg./ml., a ceiling response was produced (Fig. 6). A concentration of 500 mcg./ml. of I produced a total paralysis of the jejunum which, upon washing, was restored to the normal control rhythm and force.

The actions of I were examined on the spontaneously contracting rabbit jejunum, on the acetylcholine-stimulated jejunum, and in the presence of barium chloride as a nonspecific stimulator for the jejunum. Control pendulum contractions of 13/min. were reduced to an average of 8.5 contractions/min. with 50 mcg. I/ml. bath, to 8.2 contractions/min. with 100 mcg. I/ml. bath, and to 8.0 contractions/min. with 200 mcg. I/ml. The average control gram-force was reduced to a 0.91 gram-force with 50 mcg./ml. and to 0.85 gram-force at 100 mcg. I/ml. and to the same average with 200 mcg. I/ml. (Figs. 7 and 8). Stimulating the jejunum with 0.01 mcg. acetylcholine/ml.

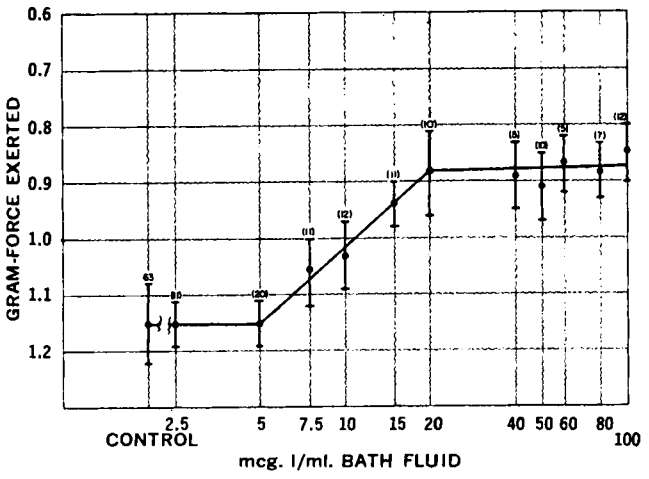

Figure 6—*Log-dose response plot of the reduction of the gram-force exerted by the isolated rabbit jejunum after incremental increases of I concentrations in the bath. A log linear response was obtained when a least-squares fit was applied for the concentration range of 5.0–20 mcg. I/ml. bath. No response was seen before 5 mcg./ml., while a ceiling effect was noted after 20 mcg./ml. The gram-force was recorded as the mean of the means with the standard deviations shown. The number of means used for this concentration is indicated by the number in parentheses.*

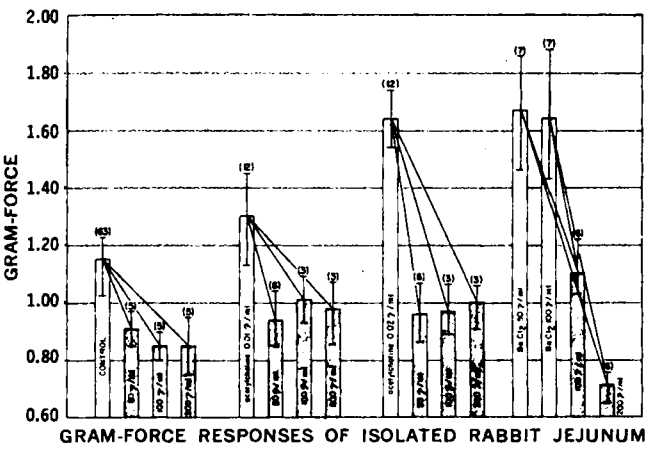

Figure 8—*Histogram of the reduction of gram-force exerted by the isolated rabbit jejunum treated with I at three different concentrations. The shaded bars represent the compound at concentrations of 50, 100, and 200 mcg./ml. of bath. The gram-force was tabulated as the mean of the means with the standard deviations shown. The number in parentheses represents the number of means evaluated for each bar. The concentrations of the two agonists are per milliliter of bath. The responses induced by the compound after barium chloride were tabulated together since no significant difference of the response to the two concentrations of barium chloride was noted.*

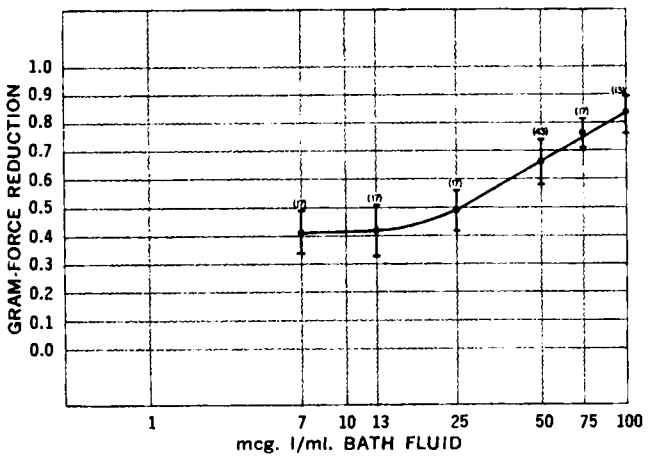

Figure 9—*Log-dose response plot of the reduction of gram-force exerted by the isolated guinea pig trachea after incremental increases of I in the bath. A log linear response was obtained with a least-squares fit applied for the concentration range of 25–100 mcg./ml. bath. No significant response was seen before 25 mcg./ml. bath. The gram-force was recorded as the mean of the means with the standard deviation shown for each incremental increase of I. The number in parentheses represents the number of means evaluated for this concentration.*

bath led to an increase in both frequency of rhythmic contraction and gram-force exerted over the control values: 17 contractions/min. and 1.30 gram-force, respectively (Figs. 7 and 8). A dose of 50 mcg. I/ml. bath in the presence of the same amount of acetylcholine led to a reduction of the frequency of the pendulum contractions to an average of 9.0 contractions/min., while a reduction of the gram-force to an average of 0.94 g. occurred. Increasing the concentration of I to 100 and 200 mcg./ml. did not produce changes significantly different from the responses produced by the addition of 50 mcg./ml. (Figs. 7 and 8).

The addition of 0.02 mcg. acetylcholine/ml. bath led to an average response of 16 contractions/min. and 1.64 gram-force. When repeating the concentrations of 50, 100, and 200 mcg. I/ml. bath, the following values for the frequency of contractions were observed: 11.0, 9.4, and 8.0 contractions/min., respectively, and gram-force response was 0.96, 0.97, and 1.00 g., respectively. The isolated rabbit jejunum was exposed to barium chloride (50 and 100 mcg./ml. bath) and a marked increase in gram-force (1.67 and 1.64, respectively) was observed. The frequency of pendulum contractions for 50 mcg. $BaCl_2$/ml. bath was not significantly different from the control value; 100 mcg. $BaCl_2$/ml. bath led to a pronounced reduction of the pendulum frequency: 2.5 contractions/min. A concentration of 100 mcg. I/ml. bath in the presence of either 50 or 100 mcg. $BaCl_2$/ml. led to a reduction of gram-force to 1.10 g. Using 200 mcg. I/ml. bath in the presence of either 50 or 100 mcg. $BaCl_2$/ml. produced a reduction to 0.72 gram-force; 100 mcg. I/ml. bath produced 5.0 pendulum contractions/min. in the presence of 50 mcg. $BaCl_2$/ml., while 200 mcg. I/ml. produced 7.2 contractions/min. in the presence of 50 mcg. $BaCl_2$/ml. No pendulum contractions were measured after 100 mcg. $BaCl_2$/ml. since the jejunum went into spasmodic contracture even after a small amount of I (25 mcg. I/ml.) (Figs. 7 and 8). The reductions of the pendulum contractions and gram-force after the addition of I to the bath could be counteracted by the addition of acetylcholine or the removal of I from the bath by washing. Normal control values for both the contractions per minute and the gram-force could be restored.

Effects of β-Hydroxylamine Cinnamonyl Hydroxamic Acid on Isolated Guinea Pig Trachea—The minimum amount of I required to produce a degree of relaxation in the rather quiescent guinea pig trachea was 25 mcg. I/ml. bath. Increasing the concentration of I from 25 to 100 mcg./ml. bath produced a linear log dose reduction of the gram-force exerted by the guinea pig trachea (Fig. 9). No pendulum contractions could be measured in this organ system. The average control value for the gram-force of the trachea was 1.6 g. This could be reduced to an average of 0.94 g. or a reduction of 0.66 g. from the control values after the addition of 50 mcg. I/ml. bath. The addition of 8 mcg. histamine hydrochloride/ml. bath produced a sustained contraction of the trachea (1.82 gram-force), which could be antagonized with a dose of 50 mcg. I/ml. bath. Introducing 50 mcg. I/ml. to the bath fluid before the addition of histamine (8 mcg./ml.) diminished the histamine response by an average of 0.76 gram-force. In this case, I behaved much as an antihistamine would by inhibiting the histamine response (Fig. 10).

A similar observation was made when acetycholine was substituted for histamine as the agonist. The addition of 1 mcg. acetylcholine/ml. bath increased the force to 1.78 g., and this could be antagonized with 50 mcg. I/ml. bath to reduce the force to 1.12 g. Placing 50 mcg. I/ml. into the bath before the addition of acetylcholine diminished the acetylcholine response by an average of 0.68 gram-force. Relaxation of the trachea could be achieved with I when the organ had been pretreated with dichloroisoproterenol followed by histamine stimulation and β-block challenge with isoproterenol. The gram-force produced during the histamine stimulation was 1.82 g., while isoproterenol (0.08 mcg./ml. bath) produced a reduction of 1.40 g. which did not differ from the control value. When this preparation was followed with 50 mcg. I/ml., a reduction to 0.88 g. could be observed. Stimulating the trachea with histamine (8 mcg./ml.) produced a force increase of 1.82 g. Dichloroisoproterenol (10 mcg./ml.) in the same bath led to a moderate reduction of force of 1.66 g. Adding 50 mcg. I to this preparation produced a reduction of force to 1.00 g. Diphenhydramine (10 mcg./ml.) inhibited the histamine response to an average value of 1.44 gram-force. Adding 50 mcg. I/ml. to this preparation produced a reduction of 0.40 gram-force (Fig. 10).

Calcium Analysis of Organ Bath in the Presence of β-Hydroxylamine Cinnamonyl Hydroxamic Acid—To exclude the possibility that I may interfere with the availability of ionic calcium in the bath and, therefore, inhibit contractions, a series of calcium anal-

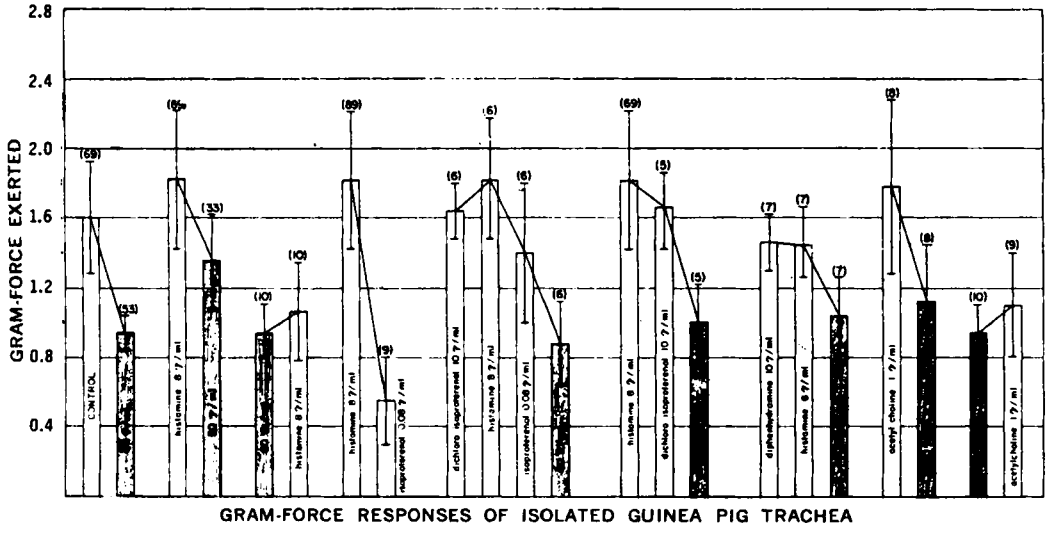

Figure 10—*Histogram of the reduction of gram-force exerted by the isolated guinea pig trachea after treatment with I (50 mcg./ml. bath; shaded bars) in the presence of agonist and blocking agents. The gram-force was tabulated as the mean of the means with the standard deviation shown for each tabulation. The number in parentheses represents the number of means evaluated for each condition studied. The concentrations for the agonists and blocking agents are expressed as per milliliter of bath.*

yses was carried out on organ bath preparations of the atria. Table I reveals no difference in values for the control concentrations of calcium and the calcium concentrations in the bath when I was present. By using ethylenediaminetetraacetate disodium salt as a chelating agent, ionic calcium was removed from the bath in amounts equal to 25, 50, 75, or 100% of the total. This produced morphologically distinct different relaxation in the atria than I produced. While I produced a contracture of the atria at higher concentrations, ethylenediaminetetraacetate produced a total relaxation with paralysis at 100% removal of calcium ion.

Table I—Ionic Calcium Analysis (3)[a]

	Control, mg. Ca^{+2}/ 50 ml. Bath	1 mg. I/ml. Bath, mg. Ca^{+2}/ 50 ml. Bath
	3.9	4.1
	4.2	4.1
	4.1	4.2
	4.5	4.4
	4.4	4.4
	4.3	4.2
	4.1	4.3
	3.7	3.5
	3.9	4.4
	4.3	4.3
	4.7	4.1
	4.3	4.3
Average[b]	4.2 mg. Ca	4.2 mg. Ca

[a] The calculated theoretical calcium concentration in the Locke solution used for the rabbit atria based on the addition of anhydrous $CaCl_2$ for the preparation of the solution should be 4.3 mg./50 ml. bath. Both the control and experimental values represent the mean of three samples in a series of 12 analyses from 12 different experiments with the atria. [b] The average values represent 36 control and 36 experimental analyses.

DISCUSSION

These isolated organ experiments clearly show that β-hydroxylamine cinnamonyl hydroxamic acid (I) produced inhibition of autogenous contractile mechanisms. This inhibition was, however, not limited to the autogenous drive but was also active in the presence of an agonist that magnified the autogenous contractile drive. It became evident that this inhibition of the contractile drive was not limited to one particular mediator system. The degree of inhibition was related to the concentrations of I in the bath as well as to the strength of the autogenous contractile drive. In the atria, an organ system with a very strong autogenous contractile mechanism, the minimum concentration of I to show any degree of inhibition was 400 mcg./ml. bath. This is thought to be a high concentration when compared with the amount of I needed for inhibition of the jejunum and guinea pig trachea, organs with a much weaker autogenous contractile mechanism.

The inhibition of the contractile mechanism by I was, in all cases, reversible by washing the organ preparation and, therefore, by removing I from the bath as well as from the hypothetical receptor site. The inhibitory effect of I could also be neutralized in the case of the atria with epinephrine or histamine, both potent agonists (4) for the atria that enhance its contractile force and frequency (Figs. 3 and 4).

With the rabbit jejunum, the minimum amount of I needed to effect a reduction of both the force and the frequency of the spontaneously occurring pendulum contractions was 7.5 mcg. I/ml. Inhibition of these parameters by I could be counteracted by acetylcholine or barium chloride. Normal control values could be restored by washing the jejunum and removing I from the bath fluid. The apparent smaller amount of I needed to induce some degree of inhibition in the jejunum in comparison to the atria is to be related to the much stronger spontaneous autogenous drive of the atria in contrast to the weaker drive in the jejunum. For the compound to be pharmacodynamically active, the internal contractile drive mechanism must be overcome; since the atria exhibit a much greater force and frequency of autogenous drive, the amounts of I to inhibit this drive would logically be larger than in the jejunum. In the jejunum, I produced a ceiling effect over a wider concentration range in contrast to the atria. While the atria underwent contracture after the dose of 2.00 mg. I/ml. bath had been reached, the jejunum had a latitude of 50–500 mcg. I/ml. bath before paralytic contracture occurred. In the case of the gram-force, a dose range of 20–500 mcg./ml. bath was needed for the jejunum.

Pretreatment of the jejunum preparation with acetylcholine (0.01 or 0.02 mcg./ml.) produced an increase in contractile frequency and force. When acetylcholine was followed by the introduction of I into the same bath fluid, a notable reduction of force and contractile frequency could be recorded. This indicates that I was able to interfere with either hormonally induced or spontaneously occurring contractions. The addition of barium chloride (50 mcg./ml.) to the bath produced a marked increase in the force of contraction while no significant change in frequency occurred. The addition of I to the bath in the presence of barium chloride did lead to a reduction of both frequency and force. The ability of I to antagonize the direct stimulation of the jejunum by barium chloride would indicate that I was interfering with the energy-generating machinery of the cell.

The reduction in the force of contraction of the guinea pig trachea produced by I was counteracted by the addition of either histamine (8 mcg./ml.) or acetylcholine (1 mcg./ml.). Conversely, I was able to reduce the histamine or acetylcholine-induced increases in force, behaving, in the case of the histamine stimulation, not unlike isoproterenol. However, when I was placed into the bath prior to the addition of histamine, the response produced by histamine or acetylcholine was suppressed. Moreover, I was able to produce a reduction of force in the presence of dichloroisoproterenol and diphenhydramine, indicating that the actions of I are not through any of the known mediator systems operating within tracheal tissue.

The persistent actions of I in the presence of a β-block (dichloroisoproterenol) and a histamine block (diphenhydramine) are not limited to the guinea pig trachea, since similar observations were made with the rabbit atria. As with the trachea, I was able to exert its depressing action on the force and rate of contractions of the atria in the presence of a β-block, in the presence of an α-block (phenoxybenzamine), and in the presence of atropine. These observations are in agreement with the earlier observed and postulated mode of action of I (1).

The maintenance of active contractile force in any tissue is dependent on the availability of the enzymes involved in an intact ATP system (5–7). It is suggested that I interferes with this enzyme system and, therefore, reduces the energy available to the cells for contraction. These enzymes are mostly bound to the cell membrane (8) and it is postulated that it is here that I exerts its pharmacodynamic actions, possibly by interfering with conformational changes in the receptor enzyme protein system needed to propagate the energy impulse.

Compound I did not reduce the calcium concentration in the bath and, therefore, did not change the availability of calcium to the tissues. Neither was the calcium concentration increased, excluding calcium extraction for the organ preparation. However, alteration of the calcium pump at the sarcoplasmic level so necessary for the generation of the contractile forces was not excluded.

Fishbein and Carbone (9) stated that hydroxyurea was converted to acetohydroxamic acid and that the hydroxamic acid diminished oxidative phosphorylation with a reduced ATP content in the mitochondria. In other work, it was reported (10) that hydroxyurea prevents incorporation of labeled phosphor into DNA. Since Fishbein and Carbone (9) reported that the hydroxyurea was converted to acetohydroxamic acid and Coutts (11) reported that hydroxamic acids were preferentially reacting with the coenzyme pyridoxal phosphate, it is possible that the inhibition shown by I was interference with phosphate transfer necessary for energy generation of a contraction. Coutts also reported that hydroxylamine cleaves the acetyl coenzyme A and that this leads to a cellular as well as a mitochondrial inhibition of ATP. Since I possesses a hydroxylamine group in the β-position, it is probable that not only the hydroxamic acid moiety is responsible for the pharmacodynamic action, but the hydroxylamine group may aid in the production of the muscle relaxation characteristic of I. The observations that I exerted its inhibitory action in the presence of various agonists, in different tissues and in the presence of blocking agents, would indicate that the relaxation was a result of inhibition of a common energy system.

It was reported (12) earlier that decanohydroxamic acid possesses papaverine-like, antiacetylcholine-like, and antihistamine-like ac-

tions. It may be that the generic group (hydroxamic acids), due to their peculiar molecular structure such as the R—C(=O)—N(—H)—OH moiety, are a group of oxidative phosphorylation uncoupling agents and compete for the energy available within the cellular contractile mechanism.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 14, 1972, from the *Department of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501*

Accepted for publication November 13, 1972.

Supported in part by an institutional project grant from the American Osteopathic Association.

The author gratefully acknowledges the encouragement and counsel of Dr. Elliott Lee Hix during this work and in the reivew of the manuscript.

NMR Study on 1,2,4,5-Tetrasubstituted 3,3,6,6-Tetradeuterated Cyclohexanes: Conformational Contributions and 1,3-Diaxial and Vicinal Deshielding Effects of Amino and Alkylated Amino Groups and Their Protonated Forms

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Abstract □ The partially deuterated compounds *trans*-2-*o*-tolyl-*cis*-4-hydroxy-*trans*-5-aminocyclohexanol-3,3,6,6-d_4 and *trans*-2-*o*-tolyl-*cis*-4-amino-*trans*-5-hydroxycyclohexanol-3,3,6,6-d_4, the corresponding *N*-methyl and *N*,*N*-dimethyl derivatives, the trimethylammonium iodide salts, the C-1 benzoyloxy esters of all these compounds, and a few C-4 acetoxy derivatives were investigated by NMR for conformational changes brought about by alkylation and protonation of axially oriented amino groups and for 1,3-diaxial and vicinal deshielding effects of amino and protonated amino groups. In these compounds, hydrogens H-1 and H-2 and hydrogens H-4 and H-5 make up two separate *AX* systems where, in a given chair conformation, the hydrogens of one pair have *trans*-diaxial orientations and those of the other pair have *trans*-diequatorial orientations. Changes in coupling constants J_{12} and J_{45} give a sensitive measure of conformational changes. The most pronounced conformational changes occurred with protonation of the dimethylamino derivatives in formic acid and in chloroform, where the equilibrium highly favors the inverted chair conformations with an axially oriented *o*-tolyl group. The 1,3-diaxial deshielding of the primary amino group was found to be somewhat comparable to that of the hydroxyl group, with little change in the effect upon protonation. The effects of *N*-methylation are discussed. Geminal and vicinal deshielding by the amino group and the role of *N*-methylation and protonation on these effects are given. Further data supporting the long-range deshielding by the carbon–carbon double bond and the epoxy group are also presented.

Keyphrases □ Cyclohexanes, 1,2,4,5-tetrasubstituted 3,3,6,6-tetradeuterated—NMR study, conformation contributions and deshielding effects of amino groups and protonated forms □ Coupling constants, 1,2,4,5-tetrasubstituted 3,3,6,6-tetradeuterated cyclohexanes—conformation changes produced by alkylation and protonation of axial amino groups □ Deshielding effects, 1,3-diaxial and vicinal—1,2,4,5-tetrasubstituted 3,3,6,6-tetradeuterated cyclohexanes, conformation changes produced by alkylation and protonation of axial amino groups □ NMR spectroscopy—tetrasubstituted cyclohexanes, conformation changes produced by alkylation and protonation of axial amino groups, vicinal deshielding effects of amino groups, coupling constants

A number of partially deuterated six-membered ring compounds have been prepared in this laboratory in recent years for NMR conformational analysis studies (1–4) and for study of long-range 1,3-diaxial deshielding of ring hydrogens by the hydroxyl group (5). On the basis of this previous work, the substituted 3,3,6,6-tetradeuterated cyclohexene (III) was easily available as an ideal intermediate for the synthesis of the tetradeuterated compounds of series VI–IX, of known stereochemistry, through the epoxides IV and V. The stereochemistry for IV and V was established for the nondeuterated epoxides (6). The deuterated compounds of series VI through IX were of special interest for the study of possible conformational changes as a function of solvent resulting from successive alkylation of the amino group and/or from protonation of the primary, secondary, and tertiary amines. The tetradeuterated compounds also provide useful systems for the study of the relative 1,3-diaxial deshielding properties of primary, secondary, and tertiary amino groups and the effect of protonation on this property. The series further proved useful in demonstrating the deshielding effect of protonation of the amino groups on the chemical shifts of vicinal hydrogens.