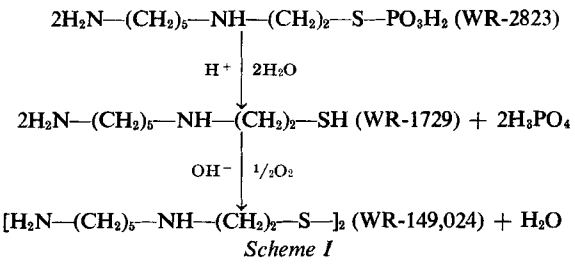
Antagonism of Norepinephrine Responses of Isolated Rabbit Aorta by Aliphatic Sulfur-Containing Amines

GALE E. DEMAREE, ROBERT E. BROCKENTON, MELVIN H. HEIFFER, and WILLIAM E. ROTHE

Abstract □ *S*-[2-(5-Aminopentyl)aminoethyl]phosphorothioic acid (WR-2823), *S*-[2-(5-aminopentyl)aminoethane]thiol (WR-1729), and 1,18-diamino-6,13-diaza-9,10-dithiaoctadecane (WR-149,024) were tested on the isolated rabbit aorta for effects to antagonize the responses to norepinephrine. The relative potency of these agents to antagonize responses to norepinephrine was WR-149,024 > WR-1729 > WR-2823. The pA_2 plot for WR-149,024 against norepinephrine gave a value of 5.47, with a slope not significantly different from −1.0. WR-149,024 did not antagonize responses to angiotensin or histamine. These findings are consistent with the hypotheses that WR-149,024 is the active form of this series of agents and that these chemicals antagonize norepinephrine responses by competitive inhibition at the α-adrenergic receptors.

Keyphrases □ Sulfur-containing aliphatic amines—α-adrenergic blockage, antagonism of norepinephrine responses, rabbit aorta □ α-Adrenergic blockage—sulfur-containing aliphatic amines, antagonism of norepinephrine responses, rabbit aorta □ Norepinephrine responses—antagonism by sulfur-containing aliphatic amines, rabbit aorta

S-[2-(5-Aminopentyl)aminoethyl]phosphorothioic acid (WR-2823) has α-adrenergic blocking properties *in vivo* as judged by the effect of the agent on the cardiovascular responses to sympathomimetic amines in several species (1). We speculated at that time that this action might be due to the hydrolysis product of WR-2823, namely, *S*-[2-(5-aminopentyl)aminoethane]thiol (WR-1729). The latter compound is oxidized in the presence of oxygen to a symmetrical disulfide, 1,18-diamino-6,13-diaza-9,10-dithiaoctadecane (WR-149,024). These three compounds are related as shown in Scheme I.



Scheme I

All three compounds have α-adrenergic blocking properties on the cardiovascular responses to sympathomimetic amines in the anesthetized rat (2). The present study was done to verify the α-adrenergic blocking properties of these agents using an isolated vascular smooth muscle and to quantitate the anti-adrenergic potency of the members of this series.

EXPERIMENTAL

WR-2823 occurred as a mixture containing 75% WR-2823, 11% water, 7% WR-149,024, and 7% inorganic phosphate (3). WR-1729 and WR-149,024 occurred as the di- and tetrahydrochlorides, respectively[1]. All materials were white granular powders with faint-to-moderate mercaptan odors; they were very soluble in water.

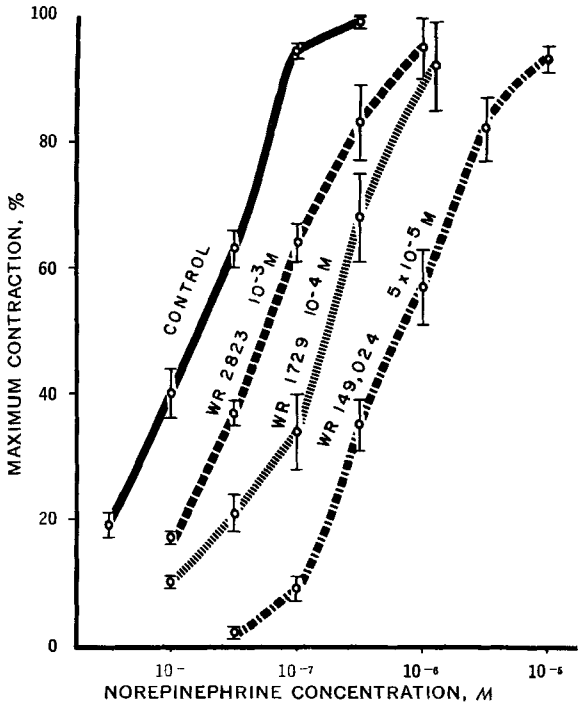


Figure 1—*Effect of WR-2823, WR-1729, and WR-149,024 on the concentration–response curve of norepinephrine on the isolated rabbit aorta. Each point represents the mean and* SEM (n = 4). *Control response is the composite of the preexposure responses. The responses after a 1-hr. exposure to each chemical were derived from separate experiments.*

Norepinephrine[2] was diluted in 0.9% saline containing 0.1% sodium bisulfite so that when added in volumes of 1 ml. in a 130-ml. muscle bath, appropriate concentrations were obtained. Histamine dihydrochloride and angiotensin amide[3] were similarly dissolved and diluted in distilled water.

New Zealand white rabbits of either sex, weighing 2.5–3.5 kg., were used. Four spiral strips of thoracic aorta from each rabbit were suspended simultaneously in an isolated muscle bath containing a balanced salt solution at 37.5°, according to the method of Furchgott (4). After a 4-hr. equilibration period at 2-g. resting tension, cumulative concentration–response curves to norepinephrine were run. The bath was drained and filled with fresh bathing solution three times; the test chemicals were then added as solutions in distilled water to the bath at various concentrations for various time periods. With the chemicals still in the bath, cumulative concentration–response curves to norepinephrine were repeated. The ED_{50} was estimated before and after the test drug was added. The $\log_{10}$ of the ratio of these two estimates (after:before) was assessed as a function of time and concentration to compare the potency of the drugs. The pA_2 for WR-149,024 was estimated by the method of Schild (5) using a 90-min. contact time. The effect of WR-149,024 at

[1] All chemicals were synthesized by Dr. W. Gannon, Regis Chemical Co., U. S. Army Contract No. DA-49-193-MD-2590.
[2] Levophed.
[3] Hypertensin.

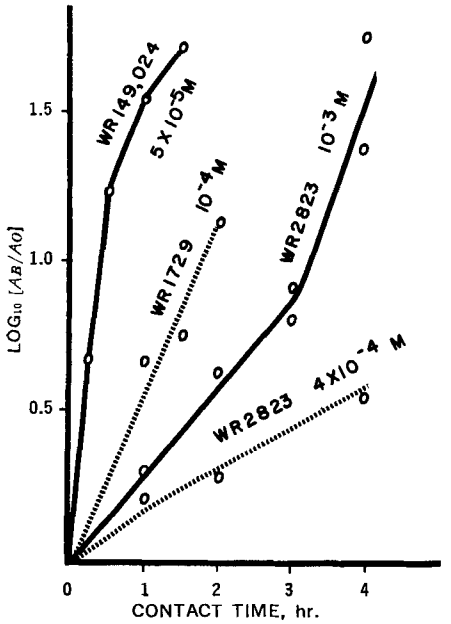


Figure 2—*Time course for the onset of the antagonism of the response of the isolated aortic strip of rabbits to norepinephrine by WR-2823, WR-1729, and WR-149,024. Key:* A_B = *concentration of norepinephrine required to give 50% maximum response in the presence of the antagonist, and* A_O = *concentration of norepinephrine required to give 50% maximum response in the absence of antagonist. Each ratio represents the results of a paired experiment from four strips.*

5×10^{-5} *M* for a 90-min. contact time on the responses of the aortic strips to histamine and angiotensin was similarly evaluated.

RESULTS

The effects of 1-hr. exposure to WR-2823, WR-1729, and WR-149,024 on the response of the isolated rabbit aorta to norepinephrine are shown in Fig. 1. Each chemical caused a parallel shift of the concentration–response curve to the right. The time course for the onset of antiadrenergic effects of WR-2823, WR-1729, and WR-149,024 is shown in Fig. 2. The onset of action and the potency of WR-149,024 were greater than those of the other two chemicals of this series. WR-1729 had a faster onset of action and greater potency than WR-2823.

The plot of the pA_2 estimate for WR-149,024 at 90-min. contact time is shown in Fig. 3. The pA_2 estimated from the least-squares

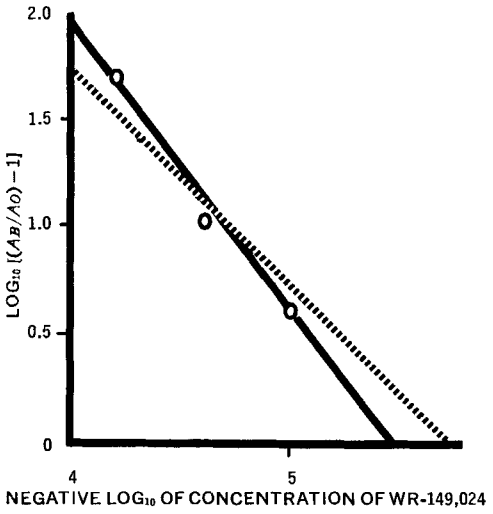


Figure 3—*Plot for the estimate of the* pA_2 *for WR-149,024 using a 90-min. contact time against the responses to norepinephrine. (For explanation of symbols, see Fig. 2 and text.) Key: —, slope of −1.31; and - - - -, slope of −1.0.*

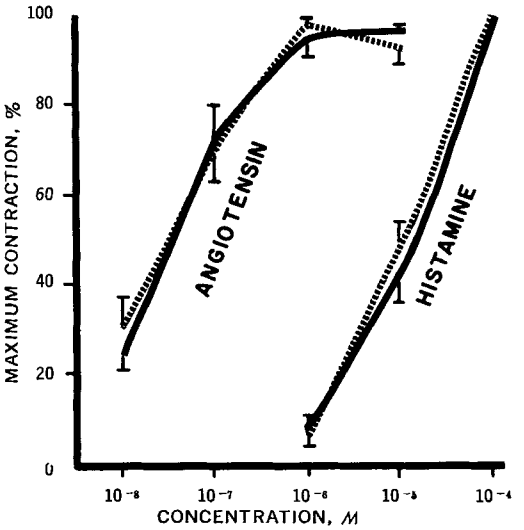


Figure 4—*Effect of WR-149,024 on the responses of isolated aortic strips to histamine or angiotensin. Each point represents the mean and* SEM (n = 4). *Key: —, control responses; and - - -, responses after a 90-min. contact with* 5×10^{-5} M *WR-149,024 in the bath.*

slope of −1.31 is 5.47; the pA_2 estimated from a slope of −1.0 is 5.73.

Responses to histamine and angiotensin were unchanged following a 90-min. exposure to 5×10^{-5} *M* WR-149,024 (Fig. 4).

DISCUSSION

The order of potency of antagonism of norepinephrine by this series of compounds is WR-2823 < WR-1729 < WR-149,024. The order of the rate of onset of antagonism is the same, with WR-2823 reaching its maximum potency most slowly, WR-149,024 reaching its maximum potency most rapidly, and WR-1729 being intermediate in rate of onset. There are two possible explanations for these two observations. These findings could represent differences in diffusion rates of the chemicals to the receptor sites, or they may be due to a conversion of the phosphorothioate to the thiol and the oxidation of the thiol to the disulfide, with this latter compound being the actual active form of this series. These experiments do not rule out either explanation, but the observation that WR-2823 had not reached equilibrium with the receptors even at 4 hr. would certainly represent an unusually slow diffusion rate. At this time, the explanation involving chemical hydrolysis is favored.

WR-149,024, the most potent member of the series, does not antagonize the responses to angiotensin or histamine at a concentration and contact time that depress the responses to norepinephrine by about 70-fold. This finding shows that the antagonism is specific for adrenergic mechanisms. The plot of the pA_2 estimate gives a line whose slope, within experimental error, is not different from −1.0. These data do not rule out other explanations, but they are consistent with the concept that WR-149,024 acts to block adrenergic mechanisms at the level of the α-adrenergic receptors as an equilibrium antagonist.

WR-2823 and WR-149,024 were shown to have efficacy in the prevention of mortality from shock due to hemorrhage, endotoxin, or anaphylaxis in several species (6–11). These chemicals are being proposed for clinical trials for therapy of endotoxin or hemorrhagic shock. If the efficacy of these compounds in this proposed use is dependent upon their ability to block peripheral adrenergic responses, the disulfide should clearly be superior to the phosphorothioate owing to its greater potency and more rapid onset of action.

All commonly used α-adrenergic blocking agents have cyclic components. The possibility that this series of aliphatic amines has α-adrenergic blocking properties raises a question concerning the requirement for aromatic configuration for α-adrenergic blockade.

These experiments provide no insight into the possible role of the sulfur components of these chemicals with respect to interaction with the adrenergic receptors. Nevertheless, they raise the interesting possibility that these agents may act by forming a mixed disulfide with the receptors or with some other cellular component at an "allosteric site."

CONCLUSION

These findings are consistent with the hypotheses that these aliphatic amines act to antagonize adrenergic responses by competitive inhibition of the α-adrenergic receptors and that the active form is 1,18-diamino-6,13-diaza-9,10-dithiaoctadecane. If these hypotheses are correct, they suggest new ideas concerning the need for aromatic structural components for α-adrenergic receptor blocking drugs.

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Hexachlorophene-Induced Changes in Electrical Response Specificity of Human Finger Epidermis for Sodium and Potassium Ions

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Abstract □ Electrical potentials developed at a human epidermal surface–aqueous solution boundary in response to varying concentrations of sodium and potassium ions were measured *in vivo*, both in the presence and absence of hexachlorophene in the solutions. The results provide further evidence for a previously postulated mechanism of hexachlorophene interaction with epidermal colloids which implicated allosterically effected changes in the electron densities of ionogenic groups vicinally located to hexachlorophene interaction sites.

Keyphrases □ Hexachlorophene—role in changes in electrical response specificity for sodium and potassium ions, human finger epidermis, mechanism of action □ Epidermis, electrical response specificity—hexachlorophene-induced changes and its mechanism of action

In a recent article concerning the interaction of hexachlorophene with human finger epidermis (1), a molecular mechanism of interaction was postulated whereby unionized hexachlorophene molecules and/or anions hydrogen bonded to peptide linkages of the epidermal proteins to effect changes in the net fixed-charge density on the surface. An inductive effect, which allosterically altered the dissociation constants of ionogenic side groups located vicinally to hexachlorophene interaction sites, was implicated as operative in the observed phenomena. Ling (2) pointed out that such changes in proton-ionization constants of ionogenic groups can also induce an altered selectivity of the ionogenic groups for associating specific monovalent cations.

In accordance with Ling (2) and Eisenman (3), the electrical response of cation-selective electrodes to specific ions is dependent upon the anionic field strength of the groups fixed to the electrically responding surface; the field strength underlies the association affinity of the surface groups for specific cations and determines the rank order of ion selectivity manifested in the electrical response behavior of the surface. Therefore, provided the magnitude of the effect is sufficient for detection, the interaction of hexachlorophene with the epidermal surface colloids may be expected to alter inductively the rank order of their selective electrical response to cations in solution.

EXPERIMENTAL

Electrical potentials were measured, utilizing the previously described experimental arrangement (1), for solutions containing HCl, NaCl, and KCl ranging in concentration from 1.0 to 10^{-5} *M*, both in the absence and presence of saturated concentrations of hexachlorophene. The previously described (1) pretreatment of fingers was employed, with the exception that prehydration was carried out in double-distilled water to effect a removal of ions from the epidermal surface. The reference solution was 1.0 *M* KCl in all instances. Measurements were initiated at both high and low concentrations and were recorded only after the potentials remained constant for a minimum of 30 sec. All NaCl and KCl solutions had pH values above 5.75. Six replications were performed on a 25-year-old male volunteer.

RESULTS AND DISCUSSION

The mean values of electrical potentials observed when the measurements were initiated at high and low ion concentrations are plotted as a function of the negative logarithm of the cation concentration in Figs. 1 and 2. The initial linear portions of the curves represent a least-squares regression analysis fit to the data. The relative specificities of the epidermal proteins for interaction with the ions were computed using the following equation as presented