were difficult to analyze as bases. They were used successfully in the oxindole synthesis after distillation and without further purification.

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Irreversible Adrenergic α-Receptor Antagonism by (*R*)- and (*S*)-*N*-(2-Chloroethyl)-*N*-methyl-2-phenyl-2-hydroxyethylamine and Related Agents

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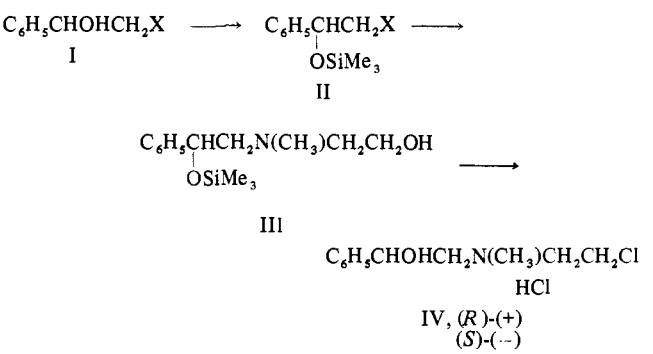
The synthesis of the title compounds is described from the corresponding mandelic acids. The compounds were significantly less effective than phenoxybenzamine as irreversible α-adrenergic receptor antagonists. The *S* isomer was more effective than the *R* isomer by a factor of 6. Interpretation of these differences is complicated by the finding that these agents, in common with many other 2-halogenoethylamines, appear to produce their actions through at least two different sites of reaction.

The 2-halogenoethylamines have been extensively employed as irreversible adrenergic α-receptor antagonists,[1,2] although their activity is not confined to this receptor system.[2] Attempts to analyze the structure-activity relationships of these agents in terms of postulated models of norepinephrine binding at the α receptor[3] suffer from a number of disadvantages.[2] In particular, the structural relationship of many of these agents, with the possible exception of the *N,N*-dimethyl-2-aryl-2-halogenoethylamines,[2,4,5] to norepinephrine seems rather obscure. Furthermore, we have recently shown that a major site of interaction of irreversible adrenergic α-receptor antagonists is at a Ca^{2+} binding/mobilization site rather than the norepinephrine recognition site.[6,7]

It thus appeared of interest to investigate compounds that are structurally more closely related to norepinephrine. Our initial investigations centered on *N*-(2-chloroethyl)-*N*-methyl-2-hydroxy-2-phenylethylamine (IV) in its enantiomeric forms. These were synthesized from optically active mandelic acids, agents of impeccable stereochemical pedigree,[8] according to the sequence shown in Scheme I.

Scheme I



Experimental Section

Melting points were determined on a Thomas-Kofler hot stage and are corrected. Analyses were performed by Dr. A. E. Bernhardt

and were within 0.4% of the theoretical values. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter and a 1-dm cell.

Phenylethane-1,2-diol Monotosylate (I, X = OTs). The optically active mandelic acids (Calbiochem Co.) were reduced with $LiAlH_4$[9] to give (*R*)- and (*S*)-phenylethane-1,2-diols [*R*, mp 66° (C_6H_6–hexane), $[\alpha]^{28}D$ −54.1° (*c* 2, Et_2O) (lit.[10] mp 66–67°, $[\alpha]^{17}D$ −47.8°); *S*, mp 66–67° (C_6H_6–hexane), $[\alpha]^{28}D$ +56.5° (*c* 2, Et_2O)] and tosylated[9] to give (*R*)-I (75%), mp 71–72° (C_6H_6–hexane), $[\alpha]^{28}D$ −32° (*c* 2, EtOH), and (*S*)-I (72%), mp 72° (C_6H_6–hexane), $[\alpha]^{28}D$ +33.2° (*c* 2, EtOH) [lit.[10] mp 73–74° (*R* and *S*)]. *Anal.* [(*R*)-, (*S*)-$C_{15}H_{16}O_4S_1$] C, H, S.

1-Phenyl-1-trimethylsilyloxy-2-bromoethane (II, X = Br). A mixture of 5 g (0.025 mol) of styrene bromhydrin in CH_3CN (25 ml) and bis(trimethylsilyl)acetamide (BSA, 2.5 g, 0.0125 mol) became warm and after 30 min hexane (50 ml) was added; acetamide was filtered off and the filtrate was stripped and distilled to give II (X = Br), bp 85° (2 mm). *Anal.* ($C_{11}H_{17}Br_1O_1Si_1$) C, H, Br.

(*R*)- and (*S*)-2-Phenyl-1-trimethylsilyloxy-2-*p*-toluenesulfonyloxyethane (II, X = OTs). These were prepared similarly to II (X = Br) to give (*R*)-II (X = OTs), mp 47–48° (hexane, 51%), $[\alpha]^{28}D$ −55.1° (*c* 2, EtOH), and (*S*)-II (X = OTs), mp 48–49° (hexane, 42%), $[\alpha]^{28}D$ +58.2° (*c* 2, EtOH). *Anal.* [(*R*)-, (*S*)-$C_{18}H_{24}O_4Si_1S_1$] C, H, S.

***N*-Methyl-*N*-(2-hydroxyethyl)-2-phenyl-2-trimethylsilyloxyethylamine (III).** II (X = Br or OTs, 0.025 mol) in 20 ml of EtOH and K_2CO_3 (3 g) and $MeNHCH_2CH_2OH$ (0.025 mol) were refluxed for 3 hr, filtered, stripped, and distilled to give (*RS*)-III (51%), bp 131° (0.04 mm), (*R*)-III (49%), bp 140–143° (0.03 mm), $[\alpha]^{28}D$ −57.8° (*c* 2, EtOH), and (*S*)-III (58%), bp 157–159° (0.1 mm), $[\alpha]^{28}D$ +60.3° (*c* 2, EtOH). *Anal.* [(*RS*)-, (*R*)-, (*S*)-$C_{14}H_{25}N_1O_2Si_1$] C, H, N.

***N*-Methyl-*N*-(2-chloroethyl)-2-phenyl-2-hydroxyethylamine (IV).** III (0.795 g, 0.003 mol) in $CHCl_3$ (10 ml) was treated with $SOCl_2$ (0.48 g, 0.004 mol) at 0° with stirring and rigorous exclusion of moisture. The mixture was warmed to 45°, stripped, and recrystallized (*i*-PrOH–Me_2CO) to give 65% of (*RS*)-IV, mp 132–134°, (*R*)-IV, mp 127–129°, $[\alpha]^{28}D$ +47.2° (*c* 3, 0.5 *N* HCl), and (*S*)-IV, mp 126–127°, $[\alpha]^{28}D$ −49.3° (*c* 3, 0.5 *N* HCl). *Anal.* [(*RS*)-, (*R*)-, (*S*)-$C_{11}H_{17}Cl_2N_1O_1$] C, H, Cl, N.

Pharmacological Testing. Vasa deferentia from albino rats (Holtzman, 150–200 g) were mounted in jacketed 10-ml organ baths containing Tyrode's solution which was maintained at 37° and aerated by a gaseous mixture (95% O_2 + 5% CO_2). Contractile responses were recorded on a smoked drum of a kymograph *via* an isotonic lever which exerted a tension of 250–300 mg and possessed a magnification ratio of approximately 1:15. Adrenergic blockade was evaluated with $10^{-4}M$ doses of phenylephrine (PE); in control experiments, $10^{-4}M$ PE caused maximal contractions of the tissue. The tissues underwent equilibration for about 30 min and the control responses to $10^{-4}M$ PE were allowed to reach steady levels before antagonistic activity was evaluated.

The adrenergic blockade induced by the test compound was evaluated by exposing the tissue to the antagonist for 10 min and determining the response to $10^{-4}M$ PE 3 min after washout of the antagonist. In experiments where the recovery of adrenergic responses was studied following maximum blockade (>90%) by the test compound, one vas deferens was treated with the test compound while the other vas deferens was treated with *N,N*-dimethyl-2-bromo-2-phenylethylamine (DMPEA, $10^{-5}M$/5 min) before being exposed to the test compound approximately 100 min later. The rationale for this procedure has been described previously.[6,7] The recovery of (maximal) responses to PE was followed for a maximum of 180 min.

Fresh solutions of drugs were made each day. The adrenergic antagonists were dissolved in warm saline and maintained at 37° for 20 min before placing them on ice. Solutions of *N,N*-dimethyl-2-bromo-2-phenylethylamine (DMPEA) were made in normal saline and immediately placed on ice.

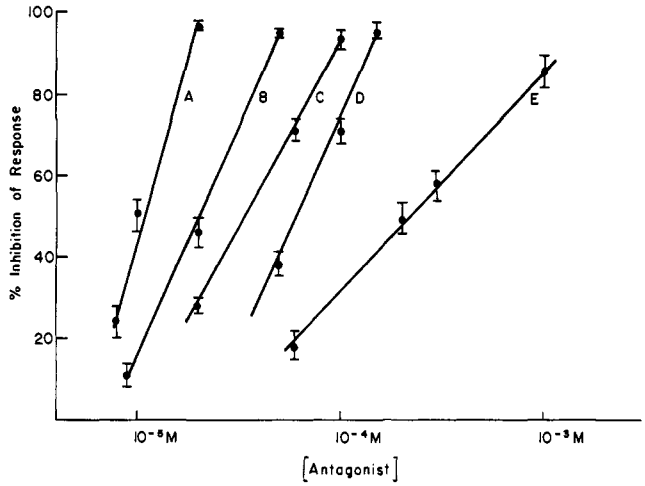


Figure 1. Adrenergic blockade caused by various concentrations of the test compounds. The vas deferens was exposed to an antagonist for 10 min; contractile response to PE ($10^{-4}M$) was determined 3 min after the washout of the antagonist. Inhibition of responses was calculated with reference to maximal control response to PE ($10^{-4}M$). Each point represents a mean of at least six observations: A = *N*-(2-chloroethyl)-*N*-methyl-2-chloro-2-phenylethylamine; B = *N*-(2-chloroethyl)-*N*-methyl-2-phenylethylamine; C, D, and E = *S*, *RS*, and *R* isomers of *N*-(2-chloroethyl)-*N*-methyl-2-hydroxy-2-phenylethylamine, respectively.

Results and Discussion

Figure 1 shows the per cent blockade produced by various concentrations of (*RS*)-, (*R*)-, and (*S*)-*N*-(2-chloroethyl)-*N*-methyl-2-hydroxy-2-phenylethylamine and by two related compounds, *N*-(2-chloroethyl)-*N*-methyl-2-chloro-2-phenylethylamine and *N*-(2-chloroethyl)-*N*-methyl-2-phenylethylamine. From the data of Figure 1, Table I has been derived showing the concentrations of the various agents required to block by 50% the response of the vas deferens to a maximum dose of phenylephrine.

Despite the very significantly lower activity of the compounds listed relative to such well-known agents as phenoxybenzamine,[11] the data do present some points of interest. Introduction of the β-OH group (**3–5**, Table I) reduces activity relative to the deoxy compound **2** and the β-chloro derivative **1** although the latter agent differs from the others reported in that it offers the possibilites of bifunctional alkylation.[12]

The (*S*)-(−) enantiomer of *N*-(2-chloroethyl)-*N*-methyl-2-hydroxy-2-phenylethylamine is approximately six times as potent as the (*R*)-(+) enantiomer when comparison is made at the concentrations required to produce 50% antagonism. Furthermore, this apparent stereoselectivity of interaction is opposite to that well established for the directly acting stimulants at the adrenergic α receptor.[13,14] The apparent loss of affinity and inversion of stereoselectivity relative to the agonists found upon introduction of the β-OH group into the antagonist species suggests that the role of this

Table I. Antagonist Activities in the Rat Vas Deferens α-Receptor System

No.	Compound	ED_{50},[a] *M*	Ratio[b]		
1	$PhCHClCH_2NMeCH_2CH_2Cl$	1.1×10^{-5}	1.0		
2	$PhCH_2CH_2NMeCH_2CH_2Cl$	2.1×10^{-5}	1.9	1.0	
3	$PhCHOHCH_2NMeCH_2CH_2Cl$ (*S*)	3.4×10^{-5}	3.1	1.6	1.0
4	$PhCHOHCH_2NMeCH_2CH_2Cl$ (*RS*)	6.0×10^{-5}	5.45	2.9	1.76
5	$PhCHOHCH_2NMeCH_2CH_2Cl$ (*R*)	2.1×10^{-4}	19.1	10.0	6.2

[a] ED_{50}: concentration of antagonist required to reduce by 50% the response of the rat vas deferens to $10^{-4}M$ PE. Values were obtained from plots of concentration–effect relationship (Figure 1). [b] Equipotent molar ratios given using 1, 2, or 3 as standard (1.0) antagonist.

Table II. Durations of Antagonist Action in Rat Vas Deferens α-Receptor System

No.	Compound	Pretreatment[a]	Max recovery (% response ± SEM)	$t_{1/2}$, min
1	$PhCHOHCH_2NMeCH_2CH_2Cl$ (*R*) ($10^{-3}M$/10 min)	Control	82 ± 7.2 at 180 min	85.4 ± 10.3
		DMPEA ($10^{-5}M$/5 min), W + 90 min	90 ± 3.2 at 60 min	22.7 ± 1.7
2	$PhCHOHCH_2NMeCH_2CH_2Cl$ (*S*) ($10^{-4}M$/10 min)	Control	90 ± 5.6 at 180 min	78.0 ± 10.7
		DMPEA ($10^{-5}M$/5 min), W + 90 min	89 ± 4.6 at 60 min	22.8 ± 2.5
3	$PhCH_2CH_2NMeCH_2CH_2Cl$ ($5 \times 10^{-5}M$/10 min)	Control	41 ± 5.7 at 180 min	>180
		DMPEA ($10^{-5}M$/5 min), W + 90 min	85 ± 4.7 at 90 min	46.2 ± 2.3
4	$PhCHClCH_2NMeCH_2CH_2Cl$ ($2 \times 10^{-5}M$/10 min)	Control	55 ± 6.2 at 180 min	~180
		DMPEA ($10^{-5}M$/5 min), W + 90 min	79 ± 2.9 at 60 min	31.0 ± 2.6
5	$PhCHBrCH_2NMe_2$(DMPEA) ($10^{-5}M$/5 min)		100 ± 1.8 at 90 min	20.7 ± 1.2

[a] Tissues were treated with DMPEA ($10^{-5}M$/5 min) to give complete blockade and 95 min later (wash + 90 min), when response to agonist was completely restored to control levels, the antagonist was added. The rationale of this procedure is discussed in ref 6 and 7.

group is not at all equivalent in agonists and antagonists. However, the concentration-effect curves for the two enantiomers are not parallel, suggesting that the measured stereoselectivity is not a single measure of stereochemical discrimination of binding at a single site.

The measured effectiveness of irreversible antagonists depends upon structural parameters controlling affinity for the macromolecular surface and upon the rate of alkylation within the preformed antagonist-macromolecular complex. For certain enzyme systems it has proved possible to separate these two parameters,[15,16] but this remains to be achieved for inactivators of pharmacological receptors. For the enantiomers of phenoxybenzamine which gave parallel concentration-blockade curves,[11] it was argued that the enantiomer potency difference reflected different affinities rather than different alkylating activities. The nonparallel curves obtained for the enantiomeric species (3 and 5, Table I) reported here may indicate that differences in affinity *and* differences in alkylating activity contribute to the observed enantiomeric potency differences. It is also possible that the observed differences reflect varying contributions of alkylation at more than one site concerned with the interference of adrenergic α-receptor mediated responses. We have recently shown that many irreversible adrenergic α-receptor antagonists, including phenoxybenzamine, dibenamine, and related compounds, exert this antagonism at two distinct sites; at one of these sites, believed to be concerned with Ca^{2+} binding, antagonism is more or less prolonged and at the second site, possibly the norepinephrine recognition site proper, antagonism is much shorter.[6,7] These sites can be distinguished by tissue pretreatment with Ca^{2+} competing species (diazoxide and local anesthetics) or by *N,N*-dimethyl-2-bromo-2-phenylethylamine, a well-investigated antagonist of short irreversible duration,[17,18] and allowing complete recovery from the latter antagonism prior to addition of the second antagonist species. Either procedure leads to an approximately equivalent conversion of a blockade of long duration to one of short duration without reduction in the degree of initial blockade produced by the antagonist species. These results clearly indicate the existence of two kinetically distinct alkylatable sites concerned with adrenergic α-receptor antagonism.

Application of this finding to the agents under discussion led to the results shown in Table II; a typical plot is shown in Figure 2. DMPEA pretreatment reduces the duration of blockade of all the antagonists listed as can be seen very clearly from the first-order plots of recovery of response (Figure 2). Significant differences do not exist between the durations of antagonism of the enantiomers of *N*-2-chloroethyl-*N*-methyl-2-hydroxy-2-phenylethylamine (1 and 2, Table II) either before or after pretreatment with DMPEA

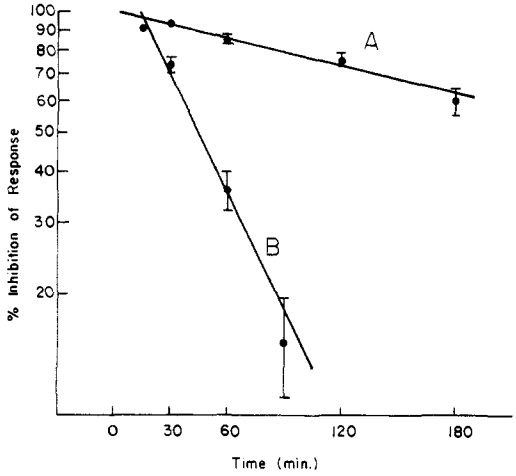


Figure 2. Recovery of adrenergic responses from blockage by $PhCH_2CH_2NMeCH_2CH_2Cl$ ($5 \times 10^{-5}M$/10 min) in nontreated tissues (A) and in DMPEA ($10^{-5}M$/5 min) pretreated tissues (B). The experimental procedures are similar to those described in Figure 1. Each point represents the mean of at least six observations.

and the extent of blockade is not sensibly altered by the pretreatment. This extension of our previous findings[6,7] of multiple sites of interaction of irreversible adrenergic α-receptor antagonists to the enantiomeric species under discussion indicates the difficulty of providing a unique interpretation of the observed stereoselectivity which may be determined by combinations of two affinity and two reactivity parameters.

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Axonal Cholinergic Binding Macromolecule. Response to Neuroactive Drugs†

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The interactions between the axonal cholinergic binding macromolecule, obtained from the walking leg nerves of the lobster, *Homarus americanus*, and several pharmacological agents which block the conduction of an axonal action potential have been studied. Those compounds which were competitive inhibitors of [^{3}H]nicotine binding included tetraethylammonium bromide ($K_i = 1.6 \pm 0.1 \times 10^{-5} M$) which blocks the increase in K^+ conductance only when applied inside of the lobster axon membrane and procaine ($K_i = 2.9 \pm 0.2 \times 10^{-6} M$) and hemicholinium 3 ($K_i = 2.8 \pm 0.2 \times 10^{-5} M$) which have local anesthetic effects and also act on the inner surface of the membrane. Ouabain was a noncompetitive inhibitor of [^{3}H]nicotine binding with a $K_i = 7.0 \pm 0.6 \times 10^{-5} M$. However, kinetic studies failed to indicate any interaction between nicotine and the Na^+K^+-ATPase. All the results still support the hypothesis that the axonal cholinergic binding macromolecule is on the internal surface of the axon plasma membrane and may be a component of both the Na^+ and K^+ gates.

Several pharmacological agents are now known which block the conduction of an axonal action potential by mechanisms which have been determined by physiological experiments.[1] It should be feasible to study the binding of such compounds to axon membranes in an attempt to identify and characterize the macromolecular components essential for axonal conduction. Such an approach is analagous to that being used to characterize postsynaptic cholinergic receptors.[2] Alternatively, it is also possible to study the interactions of these drugs and toxins with enzymes and macromolecules known to be present in the axon membrane in an attempt to assign a role to them in the conduction of an action potential. This has recently been done by Matsumura and Narahashi[3] who investigated the function of the ATPases in axonal conduction in lobster nerves.

Recently, we described a macromolecule present in an axon plasma membrane preparation from lobster walking legs which binds cholinergic ligands and local anesthetics.[4a] We shall call this the axonal cholinergic binding macromolecule (ACBM). A cholinergic receptor in the axon has long been predicted by Nachmansohn[5] who hypothesized that it plays a direct role in the conduction of an action potential along the axon; the possible relation between ACBM and this axonal receptor is of interest. In this paper we attempt to ascertain the function of ACBM by examining its interaction with some of the drugs and toxins which are known to block axonal conduction by specific mechanisms.

Experimental Section

Materials and Methods. The axon plasma membrane preparation was purified from the microsomal fraction of a hypotonic extract of the main sensory-motor nerve bundle from the eight walking legs of 1.5-lb lobsters, *Homarus americanus*. The details of this preparation and some of its characteristics have previously been described.[4]

The pharmacological agents tested and their sources are: *N*-acetylimidazole (Sigma); veratrine, mixture of alkaloids (Sigma); DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (Geigy); tetrodotoxin (Calbiochem); tetraethylammonium bromide (Eastman); procaine (Mann); hemicholinium 3 (Aldrich); phenobarbital (gift from Dr. C. Wilkinson); grayanotoxin I (gift from Dr. I. Yamamoto); and ouabain (Nutritional).

The binding of [^{3}H]nicotine (Amersham, specific activity 355 mCi/mmol) to the membrane preparation was measured by equilibrium dialysis. Aliquots of 0.35 ml containing 1–2 mg of protein/ml of the membrane preparation were dialyzed for 16 hr at 4° against 100 ml of lobster Ringers [457 m*M* NaCl, 15 m*M* KCl, 25 m*M* $CaCl_2 \cdot 2H_2O$, 4 m*M* $MgCl_2 \cdot 6H_2O$, 4 m*M* $MgSO_4 \cdot 3H_2O$, 10 m*M* Tris·HCl pH 7.5] containing the radioactive nicotine and the pharmacological agent being tested. At equilibrium, samples were taken of the contents of the dialysis bag and of the outer solution and their radioactivity was measured.[6] The difference represents the amount of [^{3}H]nicotine bound. In cases where some of the drugs showed no apparent effect on nicotine binding, they were also initially added directly at the desired concentration to the axon plasma membranes prior to dialysis. This control was to make sure that the membranes came in contact with the drug and was particularly relevant to the case of DDT which adsorbs strongly to the dialysis tubing and the glass walls of the flask. An additional factor that was necessary to consider under these conditions was the effect of the pharmacological agent on the equilibrium of [^{3}H]nicotine across the dialysis tubing. A control sample was run for each drug without axon plasma membranes. In all cases, after 16 hr identical concentrations of [^{3}H]nicotine were found inside and outside the dialysis bag.

The reversibility of the binding of those pharmacological agents which affected nicotine binding was tested by incubating the axon membranes with the compounds for 16 hr as in a regular equilibrium dialysis binding assay. After this time the dialysis bag containing the membrane and the drug was removed and placed in another flask containing 100 ml of [^{3}H]nicotine without the drug. The binding was measured in the usual manner after a second 16-hr equilibration time and compared with that of a control sample handled in an identical way but not exposed to the compound being tested. All of the compounds which inhibited nicotine binding were found to do so in a manner that was 93–98% reversible.

ATPases were assayed at 21° in 0.05 *M* Tris pH 7.5 with 3 m*M* $MgSO_4$ and 1 m*M* ATP in the presence or absence of 150 m*M* NaCl and 25 m*M* KCl. The reactions were stopped with 10% trichloroacetic acid and the inorganic phosphate released was measured by the method of Baginski, *et al.*[7] This technique has the advantage of not being sensitive to inorganic phosphate produced by acid hydrolysis of ATP after the color reagent has been added, because the excess molybdate is complexed by addition of a citrate arsenite solution.

Results

A summary of the interactions between the pharmacological agents and the ACBM is presented in Table I. The binding of [^{3}H]nicotine was used as a measure of the ACBM

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