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Chemical Transformations of Xylamine (*N*-2′-Chloroethyl-*N*-Ethyl-2-Methylbenzylamine) in Solution

Pharmacological Activity of the Species Derived from This Irreversible Norepinephrine Uptake Inhibitor

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SUMMARY

Xylamine (*N*-2′-chloroethyl-*N*-ethyl-2-methylbenzylamine), a nitrogen mustard that irreversibly inhibits norepinephrine uptake, cyclizes in solution to form an aziridinium ion. The first-order rate constants for cyclization at 23° and 37° are 0.12 min^{-1} and 0.40 min^{-1}, respectively. The aziridinium ion is relatively stable at 23° but hydrolyzes at 37° with a half-time of 70 min. A dimeric compound was indirectly shown to form at 1 mM xylamine through a reaction between the parent mustard and its aziridinium ion. A similar reaction between the 2-hydroxyethylamine and the aziridinium ion does not take place at pH 7.4. The aziridinium ion, its hydrolysis product, and the dimer were synthesized to evaluate directly their effects on norepinephrine uptake in rabbit thoracic aorta. The aziridinium ion was as potent as xylamine as an irreversible uptake inhibitor, and the effects of both compounds were sodium-dependent. The dimer was a weak competitive inhibitor of norepinephrine uptake, with an IC_{50} of about 10 μM. The 2-hydroxyethylamine, at 100 μM, competitively inhibited only 20% of control norepinephrine accumulation. These results demonstrate that the aziridinium ion is responsible for xylamine's uptake blocking activity and that the other xylamine derivatives do not influence this action.

INTRODUCTION

In previous studies from this laboratory, xylamine (*N*-2′-chloroethyl-*N*-ethyl-2-methylbenzylamine) has been shown to be a selective irreversible inhibitor of the active transport of norepinephrine across the plasma membrane of noradrenergic neurons (1, 2). Continuing investigations of xylamine's pharmacological behavior have indicated that this agent may be useful for studying several aspects of the norepinephrine carrier mechanism. The potential value of this nitrogen mustard has warranted a detailed examination of its chemistry in aqueous solution. An analysis of the chemical transformations that xylamine can undergo during pharmacological experiments is essential because of the several molecular species that a 2-chloroethylamine can generate (Fig. 1) (3).

It is known that many nitrogen mustards exhibit qualitatively similar chemical transformation in solution (4, 5). 2-Chloroethylamines undergo intramolecular cyclization to yield aziridinium ions, electrophilic intermediates that can subsequently hydrolyze or react with other nucleophiles, including those present in biological tissue. It has been demonstrated for several nitrogen mustards that a dimeric piperazinium compound can be formed through a reaction between the un-ionized 2-ethylamine and its aziridinium ion (6, 7). In some cases, the hydrolysis product (the 2-hydroxyethylamine) has also been shown to react with the aziridinium ion (8). The persistence of the blockade of norepinephrine transport by xylamine suggests that the aziridinium ion mediates this inhibition because of its alkylating ability. The involvement of the aziridinium ion would be consistent with the findings of previous studies with other nitrogen mustards (9, 10).

This report examines xylamine's chemical conversions to provide evidence for the identification of the species mediating the irreversible blockade of norepinephrine uptake. Additionally, the formation of the xylamine-derived species were studied to identify those appearing in amounts that could possibly modify the observed inhibition. In association with these analyses, we have synthesized the relevant xylamine derivatives to examine directly their effects on norepinephrine transport. Comparison of the results obtained for these compounds with xylamine's properties should unequivocally identify the biologically active species.

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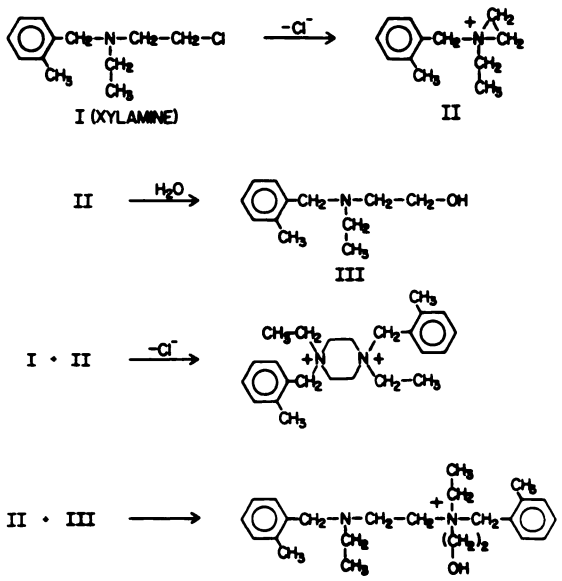

FIG. 1. *Possible chemical transformation of xylamine in aqueous solution*

METHODS

NMR spectra (200 mHz) were determined at ambient temperature on a Bruker WP 200 Fourier transform instrument using tetramethylsilane as internal reference. Chemical ionization mass spectroscopic analyses were kindly performed by Dr. Larry Gruenke on an AEI MS 902 instrument specially adapted for chemical ionization in the Department of Pharmaceutical Chemistry, University of California San Francisco School of Medicine, with isobutane as the reagent gas. Analyses were carried out by Galbraith Laboratories, Inc. (Knoxville, Tenn.). Melting points were determined on a Fisher-Johns micromelting point apparatus and are uncorrected.

The following materials were used in the study: silver perchlorate (Alfa Division, Ventron Corporation, Danvers, Mass.), sodium picryl sulfonate dihydrate (Aldrich Chemical Company, Milwaukee, Wisc.), deuterated acetone (Aldrich Chemical Company) deuterated dimethyl sulfoxide (Aldrich Chemical Company), norepinephrine bitartrate (Sigma Chemical Company, St. Louis, Mo.), L-[^{3}H]norepinephrine (New England Nuclear Corporation, Boston, Mass.), Protosol (New England Nuclear Corporation), and desmethylimipramine (Merrell National Laboratories, Cincinnati, Ohio). All other chemicals were obtained from the usual commercial sources. New Zealand White rabbits were obtained from Curd Company (Los Angeles, Calif.).

Chemical Syntheses

Xylamine hydrochloride and the aziridinium ion's hydrolysis product, the 2-hydroxyethylamine, were synthesized as described by Kammerer *et al.* (2).

Synthesis of Xylamine Aziridinium Ion

The xylamine aziridinium ion was synthesized using the method of Hinkle *et al.* (9), following the original procedure of Leonard and Paukstelis (3). Xylamine hydrochloride (0.003 mole) was added to a mixture of distilled, deionized water (30 ml) and diethyl ether (30 ml) on ice. Sodium hydroxide (0.1 N, 30 ml) was slowly added with stirring; the ether was then removed and the aqueous layer was washed twice more with ether (30 ml). The combined ether extracts were dried over sodium sulfate and evaporated under vacuum to yield xylamine base which was then taken up into dry acetone (10 ml) and cooled on ice. Silver perchlorate (0.0045 mole) in dry acetone (10 ml) was slowly added to the cold acetone solution and after 10 min at room temperature the silver chloride precipitate was removed by filtration and the acetone was removed under vacuum from the filtrate. This yielded the perchlorate salt of the aziridinium ion, an oil which was not crystallizable and was contaminated with silver salts. The oil was taken up into distilled water (50 ml) containing 0.010 mole of sodium picryl sulfonate. The aziridinium picryl sulfonate ion pair was extracted into dichloroethane (50 ml), leaving the contaminating silver salts in the aqueous phase. Ethyl ether was added to obtain yellow crystals which were recrystallized from dichloroethane with diethyl ether (m.p. 125–126°).

$$C_{18}H_{20}N_4O_9S \cdot 1/2H_2O$$

Calculated: C 45.26, H 4.43, N 11.78
Found: C 45.03, H 4.54, N 11.53

Chemical ionization mass spectroscopy (200°) was consistent with the aziridinium structure showing characteristic ion fragments at m/e 176 (the molecular ion), m/e 162, and m/e 147. NMR (deuterated acetone); δ 7.7–7.2 (m, 4H, aromatic CH), δ 4.5 (s, 2H, benzylic CH_2), δ 3.7–3.2 (m, 6H, aziridinium CH_2 and NCH_2), δ 2.5 (s, 3H, phenyl CH_3), δ 1.4 (t, 3H, alkyl CH_3). Thiosulfate titration of this product in acidic solution (see below, titration methods) showed that it consumed an equimolar amount of thiosulfate, indicating the presence of the aziridinium ion and its essential purity.

Synthesis of Xylamine Dimer

The dimer of xylamine was synthesized using the same procedure as for the aziridinium ion except that xylamine hydrochloride (0.003 mole) was used in a 2-fold excess over silver perchlorate (0.0015 mole). After the acetone was removed as above, the products (xylamine base and the aziridinium perchlorate salt) were taken up into dichloromethane (10 ml). Dimer continually precipitated from this solution over a period of 2 days as the diperchlorate salt. The small quantity of contaminating aziridinium perchlorate salt was eliminated by refluxing the precipitate in methanol for 1 hr. Cooling yielded analytically pure dimer perchlorate (m.p. 220°–221°).

$$C_{20}H_{26}N_2Cl_2O_8 \cdot 1/2H_2O$$

Calculated: C 51.43, H 6.67, N 5.00
Found: C 51.37, H 6.71, N 4.95

Chemical ionization mass spectroscopy (250°) showed ion fragments characteristic of the piperazinium structure at m/e 351, m/e 247, and m/e 143. NMR (deuterated dimethyl sulfoxide); δ 7.6–7.3 (m, 8H, aromatic CH), δ 4.9 (s, 4H, benzylic CH_2), δ 3.9 (s, 8H, piperazinium CH_2), δ 3.5 (m, 4H, NCH_2), δ 2.5 (s, 6H, phenyl CH_3), δ 1.4 (t, 6H, alkyl CH_3).

Measurement of Formation and Hydrolysis of Xylamine Aziridinium Ion

All of the chemical measurements reported represented at least three separate determinations for each experiment. At the xylamine hydrochloride concentrations used there was no change in pH on addition of the mustard salt to the buffer solution or during the period in which chemical changes were monitored. Quantitation of the aziridinium by measuring thiosulfate consumption was performed by either of two methods. The significance of the results obtained using the different methods becomes apparent under Results.

Method A. This procedure was based on that of Gill and Rang (10). A stirred 1.0 mM solution of xylamine hydrochloride was prepared in 20 mM sodium phosphate buffer (pH 7.4). At various times an aliquot (2 ml) was removed and acidified by the addition of 0.2 N acetic acid (1.0 ml) to halt cyclization. Standard 0.10 N sodium thiosulfate (0.4 ml) was added and after 20 min the unreacted thiosulfate was titrated with standardized potassium triiodide (approximately 0.002 N). The end point was visualized using a starch indicator. Hydrolysis of the aziridinium ion was also determined using this method by following its disappearance with time.

Method B. This procedure for measuring the formation of aziridinium ion differed from the above in that the sodium thiosulfate (2.0 mM) was included in the 1.0 mM xylamine solution undergoing cyclization (6). Aliquots (2 ml) were removed, acidified with 0.2 N acetic acid (1.0 ml), and titrated with the iodine solution as described above.

Chloride ion released during the cyclization of xylamine was measured with a specific ion electrode (Orion No. 94-17) in conjunction with a double-junction reference electrode (Orion No. 90-02) and using a Beckman Model 130 digital pH meter. A calibration curve was prepared by measuring the relative millivolt changes of standard sodium chloride solutions in 20 mM sodium phosphate buffer (pH 7.4). The curve was linear over chloride concentrations from 5×10^{-5} M to 1×10^{-1} M.

Tissue Studies

Experiments with thoracic aorta from New Zealand White rabbits were performed as described by Cho *et al.* (1). Xylamine hydrochloride and the aziridinium picryl sulfonate salt were prepared as 1 mM solutions in water and diluted so that 0.1 ml was added to four 2-mm (5–10 mg) aortic rings which had been preincubated in 9.9 ml of Krebs bicarbonate buffer (composition as in ref. 11) gassed with 95% O_2-5% CO_2 at 37° for 15 min. After a 1-hr incubation the rings were washed for 30 min in 10 ml of Krebs buffer at 37° to remove free drug and then transferred to 20 ml of Krebs buffer (37°) containing 0.2 μM [^{3}H]norepinephrine (specific activity 0.63 Ci/mmole). After 1 hr the rings were rinsed in ice-cold buffer, briefly blotted, and weighed. Individual rings were solubilized in 1.5 ml of Protosol tissue solubilizer overnight, and 10 ml of toluene-based scintillation fluid were added to each vial. Radioactivity was determined on a Searle Mark III liquid scintillation counter at an efficiency of 40%. This protocol is assumed to measure the irreversible inhibitory effects of xylamine and the aziridinium ion. Prolonged (90 min) wash periods after exposure to the drugs does not diminish the level of inhibition observed.

Competitive [^{3}H]norepinephrine uptake inhibition by the 2-hydroxyethylamine and piperazinium dimer was measured using the method of Takimoto *et al.* (11). These agents were prepared in water as 10 mM stock solutions and diluted so that 0.2 ml was added to 19.6 ml of Krebs buffer for each concentration studied. Four 2-mm aortic rings were preincubated in this solution at 37° for 15 min, at which time 0.2 ml of [^{3}H]norepinephrine was added (final concentration of 0.2 μM, specific activity 0.63 Ci/mmole). After 1 hr the tissue was rinsed and weighed, and its radioactivity was determined as above. Irreversible inhibition of [^{3}H]norepinephrine uptake by the alcohol and the dimer was determined as detailed above for experiments with xylamine and the aziridinium ion.

In experiments using reduced-sodium media, lithium chloride replaced sodium chloride, and lithium carbonate (with an equimolar amount of hydrochloric acid) replaced sodium bicarbonate in the Krebs buffer. Four 2-mm aortic rings were preincubated for 15 min at 37° in the appropriate low-sodium buffer prior to the addition of xylamine hydrochloride or the aziridinium-picryl sulfonate salt. After 1-hr exposure to the drugs the tissue was washed for 30 min in normal sodium Krebs buffer at 37°. Uptake of [^{3}H]norepinephrine was measured as described above. Control tissues were subjected to the same buffer conditions as the drug-treated tissues.

In all uptake experiments, net accumulation of [^{3}H]norepinephrine is defined as the difference between the observed uptake and that occurring under the same conditions but in the presence of 50 μM desmethylimipramine.

RESULTS

Transformation of xylamine in aqueous solution. The cyclization of xylamine was followed using two independent methods: measurement of chloride ion released from the 2-chloroethylamine and titration of the formed aziridinium ion by thiosulfate consumption. Figure 2 shows the time course of formation of the aziridinium ion and the maximal ion levels achieved at 23° and 37° as measured by Method A. At 37° the aziridinium ion was formed more rapidly than at 23° but it was also more labile. The half-life of the aziridinium ion at 37° was about 70 min (Fig. 4), whereas at 23° it was 20 hr at pH 7.4. The time course for liberation of chloride ion from xylamine under the same conditions is also shown in Fig. 2. Complete release of chloride ion occurred at the same time the peak aziridinium ion levels were reached.

The rate of cyclization was found to be highly pH-dependent (data not shown), and no chloride ion was liberated from xylamine at pH 5.5 after 90 min at 23°. The rate of chloride ion release increased as the pH was raised to pH 8 but did not increase thereafter. High chloride ion concentrations (150 mM)did not reduce the rate of aziridinium formation or diminish the maximal ion levels obtained at either 23° or 37°. Previous studies with tertiary 2-chloroethylamines reported the ability of added chloride ion to reduce the formation of the cyclic intermediate, presumably through back reaction of chlo-

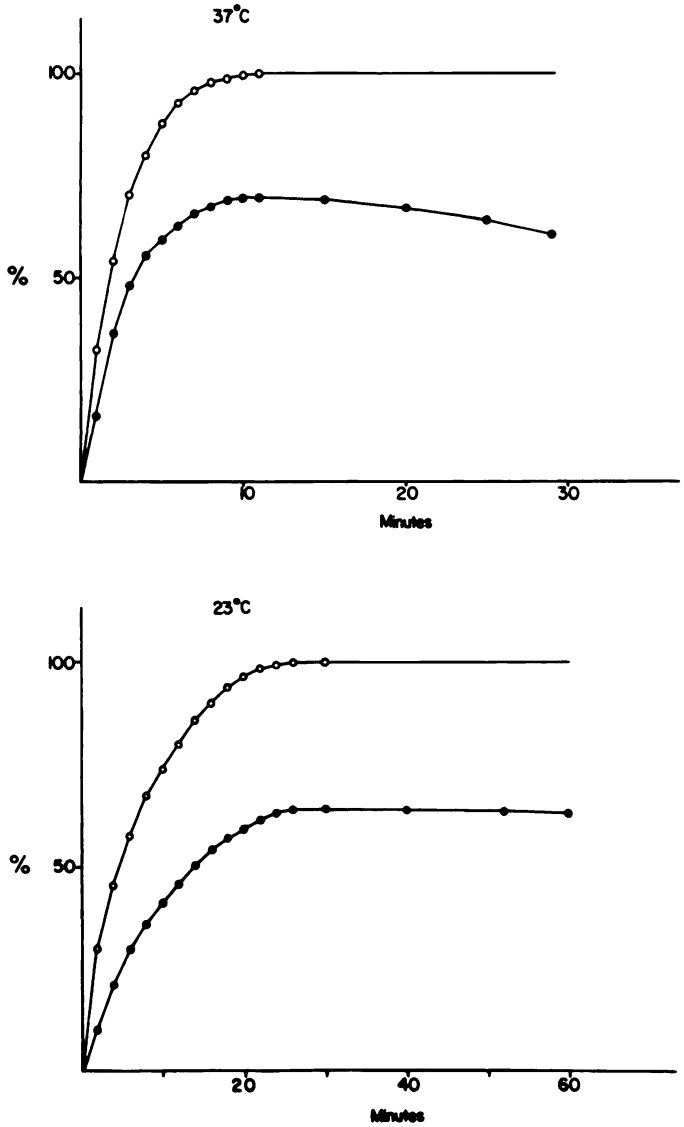

FIG. 2. *Aziridinium ion formation* (●) *and chloride* (○) *release from xylamine* (1 mM) *at* 23° *and* 37°

The ordinate expresses the amount of aziridinium ion formed or chloride ion released as a percentage of 1 Eq/mole of xylamine. The aziridinium ion was determined according to titration Method A under Methods.

ride with aziridinium ion. The rates of formation and disappearance of the aziridinium ion (6, 12) were unaffected when 26 mM sodium bicarbonate buffer (pH 7.4) was used in place of 20 mM sodium phosphate. It has been shown for other mustards that this level of bicarbonate ion can increase the rate of decomposition of the azirdinium ion (13), possibly owing to the formation of a carbonate ester that is more susceptible to hydrolysis than the aziridinium ion itself.

At 23° hydrolysis of the aziridinium ion was extremely slow. However, at this temperature, the maximal conversion of xylamine to the aziridinium ion was only 65%, although 100% of the possible chloride ion was released (Fig. 2). The residual 35% of the starting xylamine must be transformed to a species that does not consume thiosulfate but which is obviously not the 2-hydroxyethylamine. Low conversion to the aziridinium ion was noted by Harvey and Nickerson (7) in their studies with phenoxybenzamine and dibenamine, and Gill and Rang (10) observed the phenomenon in studies with benzilylcholine mustard. Both reports suggested that a dimeric piperazinium compound was being formed and was responsible for the low recoveries of aziridinium ion.

Dimerization requires the presence of aziridinium ion and the 2-chloroethylamine (Fig. 1); therefore, dimer formation might be demonstrated if the aziridinium ion could be eliminated and quantitated before it reacted with xylamine. This was accomplished by using a second titration procedure (Method B) for the aziridinium ion in which excess thiosulfate is present in solution with xylamine. Thiosulfate is a potent nucleophile and would preferentially attack the axiridinium ion and prevent dimerization. The results (Fig. 3) indicate that conversion of xylamine to the aziridinium ion is quantitative using this method. Inhibition of the dimerization of 2-chloroethylamines by thiosulfate was observed by Bartlett et al. (6) and was similarly interpreted as evidence for this bimolecular reaction. The titration method in which thiosulfate is included in solution with xylamine allows the determination of true first-order rate constants for the cyclization reaction. Aziridinium ion measurements in the presence of thiosulfate gave rate constants at 23° and 37° of 0.12 min^{-1} and 0.40 min^{-1}, respectively.

Dimerization is a second-order process, and kinetic considerations dictate that as the xylamine concentration is lowered dimer formation should be reduced as compared with aziridinium ion production. Because of the insensitivity of the thiosulfate assay and the insolubility of xylamine at much greater than 1 mM concentration, the amount of dimer present at the concentrations of xylamine (maximally 1 μM) used in the *in vitro* studies with rabbit aorta could not be determined. However, if the dimer had a high affinity for the norepinephrine uptake site, a small amount could influence xylamine's

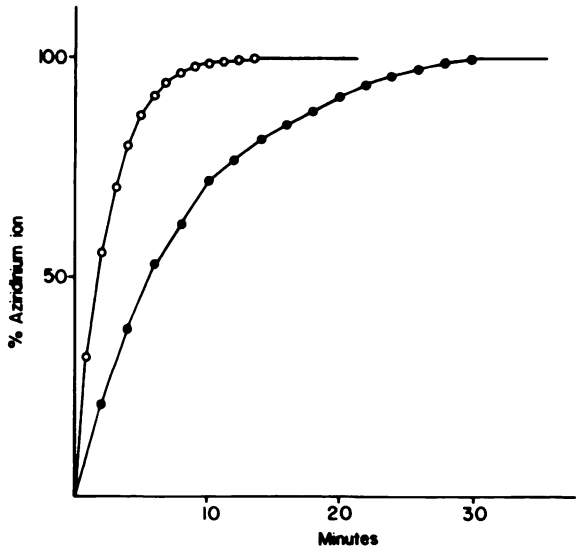

FIG. 3. *Aziridinium ion formation from xylamine at* 37° (○) *and* 23° (●) *when thiosulfate is present to prevent further reactions of the ion* (*titration Method B under Methods*)

The ordinate expresses the amount of aziridinium ion formed as a percentage of the maximum of 1 Eq/mole of xylamine.

observed inhibitory activity. For this reason, the dimer was prepared and its actions on norepinephrine uptake were evaluated directly.

At 37° the rate of hydrolysis of aziridinium ion was significant (Fig. 4), and hydrolysis occurred as an apparent first-order process with a pseudo-first order rate constant of 0.01 min^{-1}. The first-order character of the decomposition of the ion suggests that the 2-hydroxyethylamine does not act as a nucleophile by reacting with the aziridinium ion (Fig. 1). Also, it was found that at 37° the addition of the 2-hydroxyethylamine (1 mM final concentration) to an equimolar amount of aziridinium ion in solution had no effect on the rate of disappearance of the aziridinium ion. The low nucleophilicity of the 2-hydroxyethylamine may be related to its basicity since the pK_a values of tertiary ethanolamines are considerably higher than those of the corresponding chlorinated compounds (4). The higher basicity would result in a greater proportion of the cationic species being present.

In experiments using rabbit thoracic aorta the tissue was routinely exposed to xylamine for 1 hr. This relatively long exposure is required because at 1 μM xylamine, the concentration which produces complete inhibition, uptake blockade does not plateau for approximately 45 min (1). At the end of a 1-hr incubation at 37° about 30% of the xylamine would have been converted to the 2-hydroxyethylamine. Therefore the activity of this compound as an inhibitor of norepinephrine uptake was also examined directly.

Pharmacological studies with synthetic aziridinium ion, alcohol, and dimer. In these experiments segments of aorta were exposed to the aziridinium ion or xylamine and then washed to remove unbound drug before being incubated with [^{3}H]norepinephrine. Figure 5 shows that the synthetic ion and xylamine were equally effective as uptake inhibitors. Control studies with sodium picryl sulfonate showed that picryl sulfonate ion had no effect on norepinephrine accumulation.

Cho *et al.* (1) have shown that xylamine becomes less effective as an uptake inhibitor when the sodium concentration of the incubation buffer is reduced. Table 1 shows that the aziridinium ion also requires sodium for its action as an irreversible inhibitor of norepinephrine transport in aorta. This common property and the superimposable dose-inhibition curves strongly suggest that the aziridinium ion is responsible for the long-lasting antagonism of xylamine.

The results presented above indicated that the piperazinium dimer and the aziridinium ion's hydrolysis product can occur in solutions of xylamine. These compounds were synthesized to examine directly their effects on norepinephrine uptake. Since the aziridinium ion is shown to be the species mediating uptake inhibition, the studies with the 2-hydroxyethylamine and the dimer were aimed at identifying their possible modifying (i.e., antagonistic) effects on the actions of the aziridinium ion. We studied the potency of these compounds as competitive inhibitors of [^{3}H]norepinephrine accumulation in aorta, since this property would reflect any interaction they would have with the uptake carrier. The dimer was a weak competitive inhibitor of norepinephrine accumu-

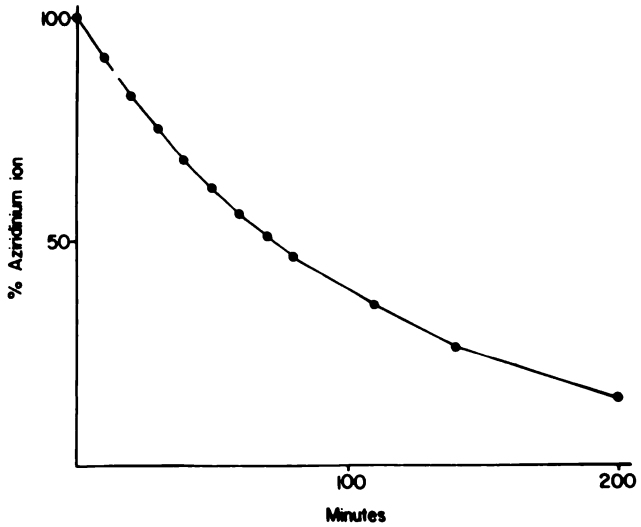

FIG. 4. *Hydrolysis of xylamine's aziridinium ion* (1 mM) *at* 37°
At each time point, an aliquot (2 ml) was quenched by adding ice-cold thiosulfate (1 ml, 0.004 N) and titrated for residual thiosulfate after 20 min.

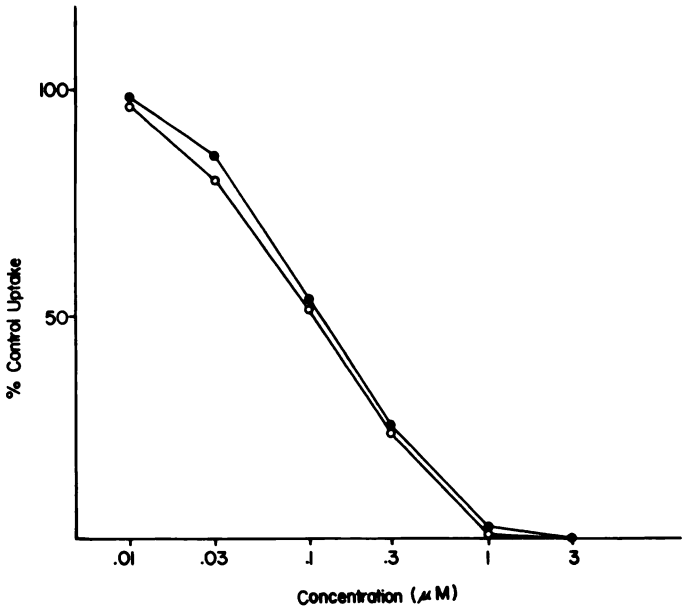

FIG. 5. *Inhibition of [^{3}H]norepinephrine uptake in rabbit thoracic aorta by xylamine (●) and the synthetic aziridinium ion (○)*
The results are expressed as a percentage of control tissue accumulation of substrate and represent three separate determinations at each drug concentration indicated.

TABLE 1

Na^+ requirement for the inhibition of [^{3}H]norepinephrine uptake by xylamine (1 μM) and the synthetic aziridinium ion (1 μM) *in aorta*

The results represent three separate determinations at each sodium concentration tested (± standard deviation).

[Na^+]	Uptake of [^{3}H]norepinephrine	
	Xylamine	Aziridinium ion
mEq	*% Control*	
140	0 ± 2	2 ± 2
70	45 ± 6	47 ± 8
0	100 ± 4	98 ± 5

TABLE 2

Competitive inhibition of [^{3}H]norepinephrine uptake by the 2-hydroxyethylamine and the piperazinium dimer of xylamine in aorta

Each concentration shows the results of three separate determinations (± standard deviation).

Concentration	Uptake of [^{3}H]norepinephrine	
	Alcohol	Dimer
μM	% control	
1.0	100 ± 5	97 ± 6
10.0	94 ± 6	59 ± 8
100.0	82 ± 4	22 ± 9

lation in aorta, with an ID_{50} of about 10 μM (Table 2). The alcohol was virtually without effect at concentrations up to 100 μM. Also, neither compound was effective as an irreversible uptake inhibitor at 100 μM when tested using the incubation protocol used for xylamine and the aziridinium ion.

DISCUSSION

This report represents one of the few times the products of a 2-chloroethylamine in solution have been identified and directly tested for their pharmacological behavior. Hinkle *et al.* (9) examined the activity of the synthesized aziridinium ions of phenoxybenzamine and dibenamine, but other investigators have relied on indirect methods for evaluating the role of the aziridinium ions derived from their respective mustards. A common approach has been to allow the 2-chloroethylamine to cyclize in solution before addition to tissue *in vitro* (13), or administration *in vivo* (14). The assumption that no 2-chloroethylamine remains may be correct, but the presence of other possibly active species has usually been ignored. Graham and Karrar (15) found that the antihistamine activity of a tertiary 2-halophenethylamine increased when the compound was kept at neutral pH for several hours at 37°. The corresponding 2-hydroxyphenethylamine was subsequently shown to be a potent antihistamine. Such a result expresses the need to examine the pharmacological activity of all of the species derived from a nitrogen mustard.

The chemical transformation of xylamine indicated that the aziridinium ion, it hydrolysis product, and the dimer should be synthesized to evaluate directly their effects on norepinephrine uptake. The aziridinium ion appeared to be the species responsible for the irreversible inhibitory action of xylamine. The alcohol and the dimer were found to be unimportant in either modifying or contributing to the aziridinium ion's activity. Xylamine cannot be involved in the observed uptake inhibition since essentially no 2-chloroethylamine remains after 10 min at 37°, and the development of inhibition is slow in aorta, requiring about 45 min for complete blockade at 1 μM xylamine.

We (1) have previously demonstrated that uptake inhibition is prevented when thiosulfate is present during the exposure of aortas to xylamine. This preliminary result suggested that the aziridinium ion is responsible for uptake inhibition, since thiosulfate does not react with xylamine. However, thiosulfate would also block formation of the alcohol and the dimer so that protection did not unequivocally prove that the aziridinium ion was the only important species. Also, a possible role for xylamine in the inhibition of transport could not be dismissed by thiosulfate's protective ability. It was not improbable that xylamine possessed the necessary affinity for the norepinephrine carrier and after binding to the uptake site xylamine could cyclize and react with a proximal nucleophile. The aziridinium ion would still be the ultimate active inhibitor in this circumstance, but inhibition would depend on the prior association of xylamine with the carrier. The activity of the aziridinium ion eliminated the possibility of this hypothetical mechanism, and it is mentioned only to indicate the interpretive limitations of protection experiments with thiosulfate.

The activity of xylamine's aziridinium ion supports the original intention in designing xylamine as an irreversible uptake inhibitor (2, 16). The aziridinium ion is a structural analogue of bretylium, a quaternary benzylamine with sympathetic neuron-blocking activity. Bretylium is a substrate for the norepinephrine transport system, and expression of its activity is dependent upon its uptake into noradrenergic neurons (17). It was proposed that the aziridinium ion should have an affinity for the uptake site as a result of this resemblance to bretylium and that it could react with a nearby nucleophile after binding to inactivate the transport system. Our studies have supported this theoretical contention. The same type of reasoning has been applied by several other investigators, for example, by Rosen *et al.* (18) in the modification of local anesthetics and by Portoghese *et al.* (19) in the synthesis of irreversible opiate antagonists. Site-directed alkylating agents such as these have a wide variety of applications and are potentially valuable pharmacological tools.

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