as the mean ± SD and tests for statistical significance were performed by using the appropriate Student's t test.

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Registry No. Codeine, 76-57-3; 3-*O*-ethylmorphine, 76-58-4; 3-*O*-propylmorphine, 74886-10-5; 3-*O*-propylmorphine hydrochloride, 86261-14-5; 3-*O*-butylmorphine, 74886-09-2; 3-*O*-butylmorphine hydrochloride, 86261-15-6; 3-*O*-pentylmorphine, 74886-08-1; 3-*O*-pentylmorphine hydrochloride, 86261-16-7; 3-*O*-hexylmorphine, 74886-11-6; 3-*O*-hexylmorphine hydrochloride, 86261-17-8; 3-*O*-heptylmorphine, 74886-12-7; 3-*O*-heptylmorphine hydrochloride, 86272-04-0; 3-*O*-octylmorphine, 74886-13-8; 3-*O*-octylmorphine hydrochloride, 86261-18-9; 3-*O*-nonylmorphine, 74886-14-9; 3-*O*-nonylmorphine hydrochloride, 86261-19-0; 3-*O*-decylmorphine, 74886-15-0; 3-*O*-decylmorphine hydrochloride, 86261-20-3; 3-*O*-dodecylmorphine, 74886-07-0; 3-*O*-dodecylmorphine hydrochloride, 86261-21-4.

Aporphines. 50.[1] Kinetics of Solvolysis of *N*-(2-Chloroethyl)norapomorphine, an Irreversible Dopamine Receptor Antagonist[2]

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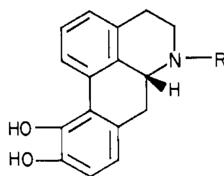
The rates and mechanism of solvolysis of (−)-*N*-(2-chloroethyl)norapomorphine (NCA, **1c**) in aqueous solution have been examined by reversed-phase liquid chromatography (HPLC) to follow the levels of starting material and products. The first-order rate constants for aziridinium ion formation at 25 and 37 °C at pH 7.0 are 0.024 and 0.096 min^{-1}, respectively. Determination of the first-order rate constant for the disappearance of NCA as a function of pH has allowed the calculation of an approximate pK_a of 6.3 for the tertiary amine, while the influence of reaction conditions (e.g., pH, buffer salt and concentration, and added nucleophiles) on product distribution support the view that NCA solvolysis proceeds through an intermediate aziridinium ion. Application of the HPLC procedure allowed us to observe simultaneously the loss of NCA and the appearance of an intermediate and multiple products at trace levels; it also permitted the facile isolation and subsequent identification of small amounts of hydrolysis products. At pH 7, maximum aziridinium concentration is reached only after 10 min at 37 °C and at 25 °C after 1 h. Increased temperatures and pH facilitate the rate of aziridinium ion formation, as well as of non-dopamine antagonist solvolysis products. The significance of these findings, including the ease with which buffer ions add to the intermediate ion, are discussed in relation to the use of NCA and its tritiated isomer, [^{3}H]NCA, in dopamine receptor studies.

The role of dopamine (DA) as a neurotransmitter at receptors in the central nervous system (CNS) and at several sites in the peripheral nervous system has stimulated intense interest in the pharmacology of DA receptors and sites of probable interactions of DA and its agonists and antagonists. Since the discovery of therapeutically useful DA agonist activity in aporphine derivatives, apomorphine (APO, **1a**) and its *N*-alkyl congener *N*-*n*-



1a, R = CH_3 [(−)-APO]
b, R = $CH_2CH_2CH_3$ [(−)-NPA]
c, R = CH_2CH_2Cl [(−)-NCA]
d, R = $CH_2CH_2OPO_3H_2$
e, R = CH_2CH_2OH [(−)-NHA]
f, R = $CH_2CH_2OCOCH_3$

propylnorapomorphine (NPA, **1b**), considerable interest has developed in the study of the interactions with DA receptors.[3–6] Such studies have been facilitated by the direct radioligand binding using high specific activity, tritiated, dopaminergic agonists and antagonists.[1,7]

Substitution of the *N*-alkyl side chain on apomorphine has a profound effect on the dopamine receptor binding and on the pharmacological effects of such aporphines.[4] Increasing the size of the *N*-alkyl substituent increases the selectivity for [^{3}H]spiperone ([^{3}H]SPR) binding sites, a property which may reflect greater lipophilicity or altered selectivity for D-2 receptors.[6]

It has been suggested that compounds containing an alkylating moiety, such as the chlorethyl group, might be useful to label membrane DNA receptor components irreversibly.[7–9] Baldessarini and his group[8] have shown that phenoxybenzamine (PBZ) can bind to DA (D-1) receptor sites in the CNS, as evidenced by its ability to block DA-sensitive adenylate cyclase activity in rat striatal homogenates. This observation has been followed up by Hamblin and Creese,[9] who reported that PBZ has a noncom-

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petitive and apparently irreversible interaction with DA receptor sites (D-2) defined by a high affinity (nM) for [^{3}H]SPR, but less so with sites (D-3) revealed by high affinity for [^{3}H]DA. A similar approach to D-2 receptor labeling is being pursued by Schuster et al.,[10] who found a long-lasting blockade of [^{3}H]SPR binding by a chloroethyl derivative of the neuroleptic compound α-flupenthixol.

The synthesis[11] and demonstration that (−)-*N*-(2-chloroethyl)-10,11-dihydroxynoraporphine [*N*-(2-chloroethyl)norapomorphine, NCA, **1c**] causes persistent DA receptor blockade suggested that this inhibition of DA receptor function may involve covalent bonding of a receptor binding site.[12-14] NCA blocked DA-sensitive adenylate cyclase activity in a noncompetitive and apparently irreversible manner.[12] This effect was prevented by coincubation with DA or APO but not with norepinephrine. Analogues of NCA with low affinity for DA receptor sites defined by binding of 1 nM [^{3}H]APO[15] also had much weaker effects against the DA-sensitive cyclase activity. These included the 10-O-methylated derivative (analogue of apocodeine) and a 10,11-diester derivative of NCA.

Additional pharmacological actions of NCA have been described.[16-20] Mustafa et al.[16] reported that NCA (57 μmol) locally injected into the nucleus accumbens of rat brain can block the behavioral excitation produced by similarly locally administered DA agonists, such as ADTN, for nearly a week. Evidence has also been presented by Lehman and Langer[17] that NCA may have reversible, DA agonist-like actions at DA autoreceptors, since it can inhibit [^{3}H]DA release from rat striatal slices in vitro and decrease levels of the DA metabolite DOPAC in mouse striatum in vivo (both effects presumably indicating autoreceptor-mediated decreases in release or turnover of DA).[17] Goosey and Doggett reported[18] that NCA appears to selectively inhibit binding of [^{3}H]APO to domperidone-sensitive binding sites, which probably represent D-2 receptors, in rat striatal tissue. The peripheral DA antagonistic effects of NCA in DA-induced renal vasodilation in the isolated perfused rat kidney[19] and in aggravating cysteamine-induced duodenal ulcer in the rat[20] have recently been observed.

These pharmacological effects of NCA may be attributed to a covalent interaction analogous to the blocking action of other β-chloroethylamines, such as PBA[21] and *N*-(2-chloroethyl)-*N*-ethyl-2-methylbenzylamine (xylamine, a

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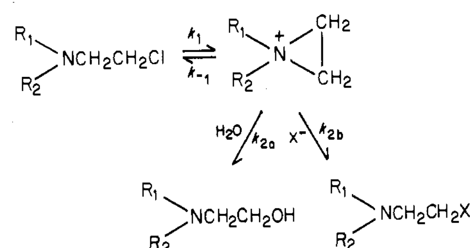
Scheme I



selective inhibitor of the membrane components associated with adrenergic receptors).[22] Several studies support the view that the alkylation of β-chloroethylamines is mediated through the aziridinium ion intermediate.[3,21-28]

Ethylcholine mustard aziridinium ion (AF 64A), a presynaptic chemical neurotoxin capable of inducing persistent deficiency in central cholinergic transmission, is an interesting example of the utility of such alkylating agents for the study of cholinergic mechanisms.[28]

The synthesis of highly reactive and chemically pure [8,9-^{3}H]NCA in our laboratories and at New England Nuclear[29] made it possible for Seeman and his group using isoelectric focusing to label macromolecular species associated with DA receptors.[30] Baldessarini and Kula[31] have carried out preliminary characterizations of the interactions of [^{3}H]NCA with a tissue preparation of the DA receptor rich mammalian basal ganglia. In view of the now strong evidence for the alkylation of DA receptors by NCA, we have undertaken a study of the stability and reactivity of NCA in aqueous solution at 25 °C (for its applicability to in vitro binding studies) and 37 °C (for in vivo studies).

Solvolysis of these compounds proceeds via a two-step process (Scheme I) in which cyclization first yields a reactive ethylenimonium ion, followed by a biomolecular ring opening by solvent or added nucleophiles. It is readily apparent that the rate of cyclization, k_1, relies on the existence of the free base of the nitrogen, and therefore, the protonated β-haloalkylamine cannot react. From this, it follows that the apparent rate of cyclization (k_{obsd}) in buffered aqueous solution is not only a function of k_1 but is a function of the dissociation constant (K_a) of the tertiary amine salt as well. In the present work we have exploited the relationship between K_a, k_{obsd}, and pH to determine k_1 for the cyclization of NCA and an approximate K_a for the base. We shall discuss the implications of the rate constants k_{2a} and k_{2b} relative to k_1 and k_{obsd} in the context of NCA interactions with DA receptors.

Several basic approaches for calculating rate constants of haloethylamine solvolytic reactions, as outlined in

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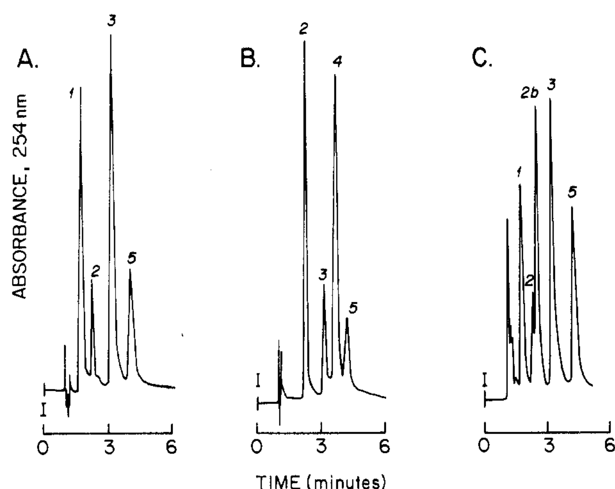

Figure 1. Chromatography of 20-μL aliquots of NCA (125 mM) reaction mixtures at 25 °C. Peaks are numbered in their order of elution, with those having the same elution time being identically numbered. Peak 1 is **1d**; peak 2 is NHA (**1e**); peak 2b is the product of NCA with *N*-acetylcysteine; peak 3 is the aziridinium ion; peak 4 is the acetate ester (**1f**); peak 5 is NCA (**1c**). The mobile phase was 80% 50 mM NaH_2PO_4 adjusted to pH 3.2 with H_3PO_4 and 20% acetonitrile. Detection was accomplished electrochemically as described in the Experimental Section. Other chromatographic conditions are also described in the Experimental Section: (A) reaction of NCA in 100 mM phosphate buffer, pH 7.0, after 65 min; (B) reaction in 100 mM acetate buffer, pH 5.8 (16 h); (C) same as in A, except with 5 mM *N*-acetylcysteine after 70 min.

Scheme I, have been employed. Other studies have used NMR to follow aziridinium formation and solvolysis.[25,26] An approach used by Henkel et al.[27] in the studies of PBA hydrolysis involves the synthesis of the reactive aziridinium ion and the independent study of each step. These authors followed cyclization with a chloride-specific electrode, while the hydrolysis of the synthesized intermediate was measured by quantitating the alcohol produced. A third technique, the one used in the present study, involves the one-step separation and quantitation of products and starting material. This approach, which has not previously been employed in the kinetic analysis of β-chloroethylamines, is best illustrated by the investigation of the acidic hydrolysis of cyclic phosphate esters by Kura et al.[32] using liquid chromatography.

Results

Cyclization of NCA to its corresponding aziridinium ion and the subsequent addition of nucleophiles to the reactive intermediate were followed by reversed-phase HPLC as described in the Experimental Section. A control experiment showed that no further reaction of NCA occurred under the conditions employed for the HPLC analysis, and estimates for the rate of ring opening by solvent and buffer ions show that only an insignificant change in the concentration of the intermediate can take place during chromatography. Typical elution profiles during the reaction are shown in Figure 1, while the kinetic profiles in phosphate and acetate buffers are presented in Figure 2. (Note that only relative peak heights are presented, not concentrations, due to the lack of standards for the aziridinium ion, the phosphate **1d**, and the acetate **1f**.) A common intermediate (open circles in Figure 2; peak 3 in Figure 1) with identical retention time is observed in each reaction medium, but only one of the stable products (× in Figure 2; peak 2 in Figure 1) had similar retention in both systems. The reaction in water yielded only this product, but in buffered systems, an additional major product was observed. The inclusion of 5 mM *N*-acetylcysteine, a good nucleophile, in a phosphate buffer reaction medium also yielded an additional product (Figure 2C; peak 2b in Figure 1C).

Table I. Effect of pH on the Rate of NCA Cyclization at 25 °C

pH	$k_{obsd} \times 10^2$, min^{-1}	$t_{1/2}$, min
5.40[a]	0.36	192
5.60[a]	0.49	141
5.80[a]	0.65	107
6.00[b]	0.97	71
6.25[b]	1.52	45
6.50[b]	1.75	39
6.75[b]	2.12	33
7.00[b]	2.43	29
7.00[c]	2.52	28
7.50[b]	2.90	24
8.00[b]	3.08	22

[a] In 0.1 M acetate. [b] In 0.1 M phosphate. [c] In 0.05 M phosphate.

Table II. Calculated Values for Kinetic Parameters by Using the Data in Table I Fitted to Equation 1[a]

program	k_1, min^{-1}	K_a	pK_a
HABELL	3.01×10^{-2}	4.91×10^{-7}	6.31
CURFIT	3.08×10^{-2}	4.51×10^{-7}	6.35

[a] If K_a = ([NCA][H$^+$])/[NCAH$^+$], then $[NCA]_{tot}$ = [NCA](1 + ([H$^+$]/K_a)) and [NCA] = $[NCA]_{tot}$/1 + ([H$^+$]/K_a). Equation 1 follows from the expression for the free base concentration.

Kinetic Analysis. The data in Figure 2 suggest that the rate of dissappearance of NCA is a function of either pH or buffer ion. Thus, a more detailed study of the rate as a function of pH was carried out, and the data were analyzed in accord with a first-order reaction mechanism (Scheme I). The amount of NCA remaining in solution at time t could be calculated by using peak heights and the linear calibration curve (Figure 3). Plots of log [NCA] vs. time showed good first-order kinetics at all pH values (Figure 4) through at least two half-lives.

In aqueous solution, NCA exists in equilibrium between the free base and the protonated form, of which only the former can cyclize. Because NCA can be quantitated in the presence of products and intermediates, the rate of NCA cyclization can be analyzed by the HPLC method independently of the subsequent ring opening step(s). Accordingly, the overall rate of aziridinium formation is not only dependent on k_1 but is also a function of the degree of ionization of the tertiary amine of NCA. Thus, it can be shown that the apparent first-order rate constant, k_{obsd}, is a function of k_1, K_a (the dissociation constant of NCA), and pH (eq 1).

$$k_{obsd} = \frac{k_1}{1 + \frac{[H^+]}{K_a}} \text{ or } \log k_{obsd} = \log\left(\frac{k_1}{1 + \frac{[H^+]}{K_a}}\right) \quad (1)$$

By determining k_{obsd} as a function of pH, k_1 and K_a can be estimated either graphically[33] or, more precisely, by statistical curve fitting with an iterative computer program. The latter approach was followed by using two different programs, HABELL[34] and a modification of CURFIT.[35] Using

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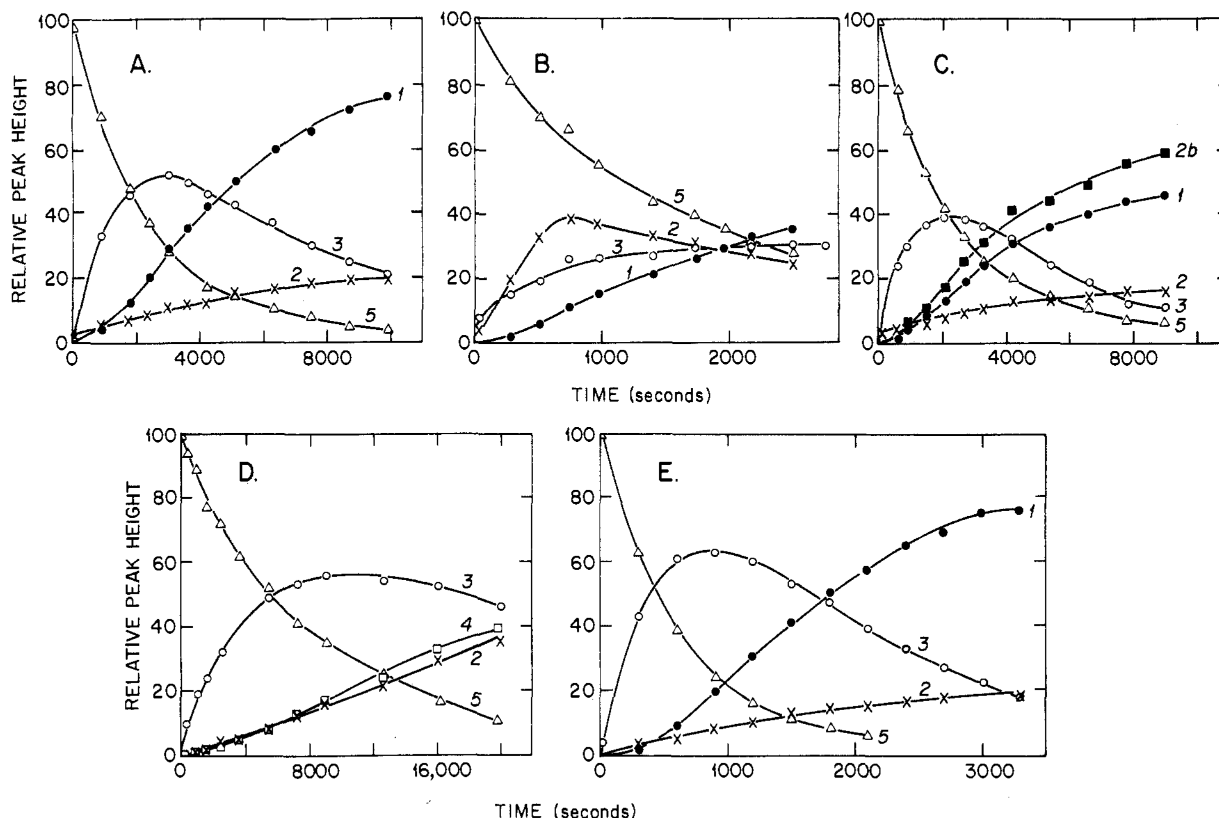

Figure 2. Kinetic profiles of NCA at 25 °C (except E at 37 °C) under various reaction conditions. The relative peak heights (NCA at 0 time = 100) are plotted as a function of time: (A) reaction of NCA in 100 mM phosphate buffer, pH 7.0; (B) reaction of NCA in 100 mM phosphate, pH 8.00; (C) reaction of NCA in 100 mM phosphate buffer, pH 7.0, containing 5 mM *N*-acetylcysteine; (D) reaction of NCA in 100 mM acetate, pH 5.80; (E) same as in A, except the temperature was 37 °C. Symbols correspond to the peaks in Figure 1 as follows: peak 1 (**1d**) (●); peak 2 (**1e**) (×); peak **2b** (■); peak 3 (○); peak 4 (**1f**) (□); peak 5 (**1c**) (△).

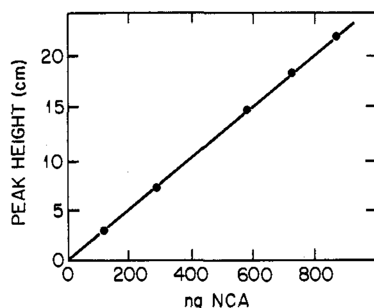

Figure 3. Calibration curve for NCA. The samples were made up in 80% 50 mM NaH_2PO_4, pH 3.2, 20% acetonitrile, and the chromatography was carried out in the same solvent. Other conditions are described in the Experimental Section.

the data shown in Table I, the two programs gave excellent agreement for k_1 and K_a (Table II) and, thus, provide a reasonable estimate for the pK_a of NCA, also shown in Table II. There is some uncertainty in this approximation of pK_a due to the effect of ionic strength differences on K_a;[36] however, no great effect of ionic strength on k_{obsd} was noted in comparing the rate in 50 and 100 mM phosphate (Table I) at the same pH.

Identification of Reaction Products of NCA Solvolysis. (A) The earliest peak (Figure 1, peak 1) is observed only when solvolysis is performed in phosphate buffers. Its electrochemical response indicates that it re-

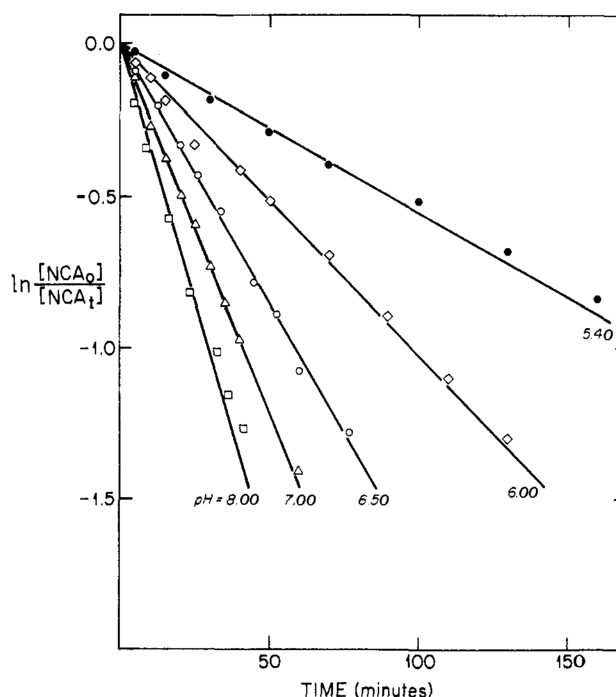

Figure 4. Kinetics of NCA cyclization at 25 °C at various pH values. Solvolysis of NCA in aqueous solution was carried out and monitored as described in the Experimental Section.

tains the catechol functionality and, therefore, is not an oxidation product; however, the compound either does not extract as an ion pair, or because of its polar nature, it does not give any mass spectrum. It was also observed that in 50 mM phosphate, pH 7.0, the rate of peak 1 production was slower than in 100 mM buffer. These properties in-

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dicate that peak 1 is a buffer addition product (**1d**), a charged phosphate.

(B) In contrast to peak 1, peak 2 is a product in all the aqueous reactions. It elutes at the retention time of authentic *N*-(2-hydroxyethyl)norapomorphine (NHA, **1e**).[11] Coinjection of the reaction mixture with authentic **1e** increased the height of peak 2 with no other observable effect. It is the only stable product of the unbuffered water hydrolysate of NCA and is generated from peak 4 (see D below). Chemical-ionization mass spectrometry yielded several prominent ions with m/e 298 [$(M + 1)^+$, base], 280, and 266, identical with authentic NHA standard.

(C) Peak 3 is also common to all studied media, but unlike peak 2, this compound is not stable. It is the most likely candidate for the aziridinium intermediate shown in Scheme I. The evidence for this suggestion is as follows: (1) it is a catechol, as determined by electrochemical detection; (2) high pH accelerates its rate of disappearance; (3) nucleophile addition to the reaction, such as 5 mM *N*-acetylcysteine, accelerates the rate of disappearance; and (4) it is a common intermediate in all NCA solvolytic reactions. Unfortunately, no interpretable mass spectrum could be obtained, not an unexpected result for a relatively involatile ionic species that is also thermally labile.

(D) Peak 4 is a product unique to solvolysis in acetate buffer. In aqueous solution it is only somewhat stable, slowly yielding peak 2 over a period of days at room temperature. The tentative identification of peak 4 as the acetate (**1f**), consistent with the above observations, was confirmed by mass spectrometry: m/e 340 [$(M + 1)^+$, base], 280, 266. In addition, on standing for several days in moist CH_2Cl_2 with CH_3CN (the organic extraction phase), a new peak appears with m/e 298, the molecular ion of NHA (**1e**), the result of hydrolysis of the ester **1f**.

(E) Peak 5 is NCA, which was confirmed by the fact that coinjection of the reaction mixture with authentic NCA increased the height of peak 5. Mass spectrometry of the picrate ion pair gave m/e 315 (M^+, electron impact) or 316 [$(M + 1)^+$, base, chemical ionization], as well as 280 and 266,[11] identical with the authentic NCA (**1c**).

Discussion

The present results support the previous hypothesis[3] that NCA in aqueous solution is an alkylating analogue of APO, and its prolonged duration of action and biochemical DA antagonism may be due to covalent modification of DA receptors. In accord with previous studies on β-chloroethylamines,[22-25] these data support the view that the solvolysis of NCA is mediated through a cyclic aziridinium intermediate.

In buffered solutions, at least two stable products are produced, the alcohol (NHA, **1e**) and the adduct of the buffer anion (**1d** or **1f**). In accordance with the findings of Henkel et al.[27] or Ransom et al.,[22] we observed no evidence for the formation of a piperazonium dimer, although our findings cannot rule out its presence entirely. However, it is apparent that nearly all of the NCA and the disappearance of aziridinium ion can be accounted for by NHA and the buffer adduct formed.

An important consideration concerning the HPLC method of kinetic analysis involves the ease with which unexpected products in complex mixtures can be identified and isolated, even at trace levels. Significant changes in product distribution were recognized easily in the unbuffered water reaction, by substitution of acetate buffer for phosphate and upon addition of the nucleophile *N*-acetylcysteine. Moreover, increasing NCA concentration to facilitate product identification is not practical because of low solubility in aqueous media, and sample handling in even slightly alkaline solution is difficult due to possible oxidation of the catechol. The use of HPLC in a moderately acidic mobile phase with subsequent extraction of the fractions as the picrate ion pair obviated the use of a neutral or basic solution that could cause decomposition of the aporphine derivatives.

The HPLC method for cyclization rate studies has also been applied to the determination of an approximate pK_a for NCA. Alternate means of pK_a measurement (e.g., titrimetric or potentiometric) are difficult to carry out due to the instability of NCA in aqueous solution, as well as its low solubility. The calculated pK_a value of 6.33 for NCA as compared to a pK_a of 7.0 for APO[37] can be rationalized as due to the increased electron-withdrawing effect of the chloroethyl group as opposed to a methyl substituent on APO.

The data in Figure 2 lead to several suggestions for DA receptor interactions with NCA and the utilization of [^{3}H]NCA for receptor isolation studies. First, with the high probability that the immonium intermediate is the species directly responsible for alkylating the DA receptor, it follows that the rate of irreversible receptor inactivation is faster in the presence of high rather than low aziridinium concentration. Consequently, those reaction conditions that produce rapid cyclization but slow displacement by solvent and buffer ions will favor covalent receptor modification. For example, although high pH (Figure 2B) results in faster NCA disappearance, the competition by OH^- for the intermediate is significant, resulting in a lower effective concentration for the alkylating agent. It can also be seen that raising the temperature from 25 to 37 °C (Figure 2A,E) greatly accelerates cyclization without a correspondingly large increase in the rate of step 2 (k_{2a} + k_{2b}). Furthermore, it is of interest to note that the favored product in the phosphate buffer system is not NHA but rather the buffer adduct, the yield of which, due to the second-order nature of ring opening, can be slowed by lowering the buffer concentration (data not shown). Another solution to the depletion of aziridinium by phosphate buffer ions would be the substitution of a poorer nucleophilic anion for phosphate (see Figure 2B for the slower acetate addition).

A second important observation with regard to DA receptor studies, using the data from Figure 2E, is that maximum alkylating rates are only reached after at least 10-min incubation ($t_{1/2}$ = 7.2 min). This is in marked contrast to studies with PBA[27] in which cyclization in aqueous solution is extremely fast ($t_{1/2}$ = 0.6 min), and the aziridinium ion concentration is probably maximal within 3 min. Hence, for in vitro studies involving receptor inactivation, the incubation time with NCA should be greater than 10 min, even at 37 °C. Furthermore, the reaction should contain a minimum concentration of nucleophiles with a pH not exceeding 7.0 in order to minimize attack by OH^-. The first-order rate constant for aziridinium ion formation at 25 and 37 °C at pH 7.0 were calculated to be 0.024 and 0.096 min^{-1}, respectively.

Independent studies, recently carried out by Baldessarini and Kula[31] using [^{3}H]NCA binding to brain tissue fractions of caudate nucleus obtained from calf brain, indicated that apparent degradation was more rapid at alkaline pH and, particularly, at a temperature elevated to 37 °C. Favorable conditions for binding assays were found at pH 6.4, 25 °C, and 30 to 60 min of incubation.[31] The slow rate of cyclization could also be a significant factor for the in vivo use of NCA. It is unlikely that the charged aziridinium ion

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could pass the blood–brain barrier (BBB), yet it has been shown that sufficient NCA can reach the CNS after peripheral administration to affect CNS dopamine receptors,[13,14] supporting the suggestion that aziridinium ion formation occurs after penetration of the BBB.

Finally, these data suggest that NCA analogues with leaving groups other than Cl^- in the β-position could have more selective properties on DA receptors. For example, a better leaving group, such as Br^- or tosylate, would lead to faster aziridinium ion formation with no change in the rate of ring opening, a situation that should result in faster receptor inactivation in vitro. However, if the drug is administered peripherally, the positively charged aziridinium ion would be poorly transported across the BBB. It is indeed possible that a poorer leaving group (e.g., F^-) would result in slower aziridinium ion formation, and, consequently, a greater percentage of administered drug would reach the CNS.

Experiments are currently underway that will test these hypotheses by the synthesis of a series of β-haloalkyl analogues of apomorphine and related structures.

Note Added in Proof: After completion of this study, Lehman, Lee, and Langer (*Eur. J. Pharmacol.* **1983**, *90*, 393) also presented evidence that NCA (**1c**) breaks down in solution into NHA (**1e**). Since NHA is more potent as a dopamine receptor agonist than NCA, it was suggested that the reversible agonistic action of NCA occurs at least partly through its hydrolysis into NHA in the perfusion medium.

Experimental Section

Materials. The aporphines NCA (**1c**) and NHA (**1e**) were synthesized as previously described.[11] HPLC-grade water and organic solvents were from Burdick and Jackson Laboratories, Inc., Muskegon, MI, J. T. Baker Chemical Co., Phillipsburg, NJ, and MCB Manufacturing Chemical Inc., Cincinnati, OH. Phenoxybenzamine (PBA) was a gift from Smith Kline & French Laboratories, Philadelphia, PA, and reagent-grade picric acid was from J. T. Baker Chemical Co. Other chemicals were reagent grade.

High-Performance Liquid Chromatography. The chromatographic system consisted of a Waters 6000A pump (Waters Associates, Inc., Milford, MA), a Rheodyne Model 7125 injector (Rheodyne, Inc., Cotati, CA), and bonded C_{18} 5 μM Hypersil silica column (150 × 4.6 mm), synthesized and packed according to a previously described method.[38] Detection was accomplished at 254 nm with an LDC Model 1203 fixed-wavelength detector (Laboratory Data Control, Riviera Beach, FL) and, occasionally, by oxidative electrochemical detection with BAS Model LC-4A detector (Bioanalytical Systems, West Lafayette, IN) using a glassy carbon electrode operating at a potential of +0.60 V.

Various phosphate mobile phases were used with Na^+ or triethylammonium as the counterion. A typical system consisted of 50 mM NaH_2PO_4 adjusted to pH 3.25 with H_3PO_4 containing 20–25% acetonitrile. Specific conditions are given in the figure captions. The flow rate was 1.5 mL/min.

Solvolysis of NCA. A 15-μL aliquot of a stock solution of NCA in methanol (2.9 mg/mL) was pipetted into 985 μL of buffer to give a 125 μM solution, and the reaction temperature was held at 25 or 37 °C in a thermostatted water bath. At measured time intervals, 20-μL aliquots were withdrawn and analyzed by HPLC. The total amount of NCA remaining was quantitated by comparing the peak height to a standard curve of NCA dissolved in mobile phase in the range 100–900 ng per injection.

The reaction buffer solution in the pH range 6.0–8.0 was a mixture of NaH_2PO_4–Na_2HPO_4. Below pH 6.0 an acetate buffer was used with 0.1 M sodium acetate titrated to the desired pH with 0.1 M acetic acid.

Identification of NCA Solvolysis Products. (1) Phosphate Buffer. NCA (1 mg/mL) was suspended in a solution of 50% methanol and 50% 0.1 M phosphate buffer, pH 7.0. After 45 min, 100 μL of this mixture was acidified with 300 μL of the HPLC mobile phase made up with 20% acetonitrile. The products were then separated by HPLC and collected in centrifuge tubes, and an aliquot of 20 mM picric acid equal to 10% the fraction volume was added to each fraction and thoroughly mixed. The resulting solution was then extracted twice with 250 μL of CH_2Cl_2, and the combined extracts were concentrated to 10–15 μL with a stream of N_2. Each fraction was then directly analyzed by electron-impact and/or chemical-ionization mass spectrometry.

(2) Acetate Buffer. A solution consisting of 1 mL of pH 5.80 acetate buffer and 200 μL of NCA in methanol (2.9 mg/mL) was allowed to stand at room temperature overnight. Under these conditions, NCA had completely reacted, and no aziridinium intermediate was present. The two remaining products were separated by HPLC in 20% acetonitrile and 80% NaH_2PO_4 buffer and then collected. Extraction and analysis by mass spectrometry were performed as described above.

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Substituted (2-Phenoxyphenyl)acetic Acids with Antiinflammatory Activity. 1[1]

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The synthesis and antiinflammatory activity of a series of substituted (2-phenoxyphenyl)acetic acids are described. Initial screening in the adjuvant arthritis test showed that halogen substitution in the phenoxy ring enhanced activity considerably. Ulcerogenic potential, as measured by the minimum ulcerogenic dose (MUD), was low in almost all the acids tested. [2-(2,4-Dichlorophenoxy)phenyl]acetic acid possessed the most favorable combination of potency with low toxicity, including ulcerogenicity, and this compound is now in therapeutic use.

A number of years ago we commenced a search for a nonsteroidal antiinflammatory drug (NSAID) that had reduced toxicity and a longer duration of action compared with existing drugs. In common with other workers,[2,3] we had noted the perhaps fortuitous resemblance of some NSAIDs to plant-growth regulators. For example, indomethacin,[4] ibufenac,[5] and a series of experimental com-

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