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3-CARBAMYL-*N*-ALLYLQUINUCLIDINIUM BROMIDE

EFFECTS ON CHOLINERGIC ACTIVITY AND PROTECTION AGAINST SOMAN

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Abstract—3-Carbamyl-*N*-allylquinuclidinium bromide (CAB) was synthesized and evaluated for its pharmacological effects on cholinergic activity and for protection *in vivo* against soman toxicity in guinea pigs. This carbamylated derivative of *N*-allyl-3-quinuclidinol (NAQ), a potent inhibitor of high-affinity choline uptake, demonstrated stereospecific alterations of cholinergic function as well as protection against soman. The *R*-isomer, but not the *S*-isomer, of CAB inhibited erythrocyte acetylcholinesterase (AChE) and plasma pseudocholinesterase (pChE) in a concentration–response manner (IC_{50} = 25 and 29 μM, respectively). The *R*-isomer of CAB was also a more potent inhibitor of high-affinity choline uptake (IC_{50} = 4.8 μM) than *S*-CAB (IC_{50} = 63 μM). When *R*-CAB (10 μmol/kg, i.m.) was administered to guinea pigs 30 min *prior* to soman in conjunction with atropine (16 mg/kg, i.m.) given 1 min post-soman, the compound significantly reduced lethality up to 5 LD_{50}s. This represents enhanced protection when compared to NAQ (up to 100 μmol/kg); the *S*-isomer of CAB failed to protect against soman intoxication. The results demonstrate that reversible inhibition of AChE with suppression of acetylcholine synthesis into a single compound, CAB, enhances the protection against organophosphates.

Intoxication by organophosphates is of concern due to their use as agricultural pesticides and potential chemical warfare agents. Soman (1,2,2-trimethylpropyl-methylphosphonofluoridate; pinacolylmethylphosphonofluoridate) is one of the most toxic organophosphonate cholinesterase inhibitors. Binding and inactivation at the active site of the acetylcholinesterase (AChE)† cause an excessive accumulation of acetylcholine (ACh), which is responsible for many of its toxic effects [1]. The inhibition is essentially irreversible.

Advances in the treatment of nerve agent toxicity have been reviewed recently [2]. Approaches to the treatment of organophosphate poisoning include: (a) post-exposure administration of atropine, a muscarinic receptor antagonist, in combination with artificial respiration and oxime reactivation of the enzyme [3–7]; (b) pre-exposure carbamate protection of the enzyme [8–13]; (c) use of benzodiazepines as anticonvulsants [14–17]; and (d) pre-exposure treatment with compounds known to suppress the synthesis of ACh [18–23]. However, these therapeutic approaches have met with only limited success in reversing soman toxicity, mostly due to the rapid "aging" process of the phosphonylated cholinesterase, which renders the enzyme insensitive to reactivation by oximes such as pralidoxime and HI-6 [4-aminocarbonyl-pyridinium-1-methyleneoxy-2′-(hydroxyiminomethyl)-1′-methylpyridinium dichloride monohydrate]. More recent studies have demonstrated that the intravenous administration of purified butyrylcholinesterase and acetylcholinesterase can effectively protect mice from multiple LD_{50}s of soman [24].

Studies in our laboratory [18] and others [19–23] have examined whether compounds known to suppress presynaptic synthesis of ACh *in vitro* would enhance the protective regimen against soman *in vivo*. Schoene *et al.* [20] and Harris *et al.* [21] found that naphthyl vinylpyridinium, a choline acetyltransferase (ChAT) inhibitor, reduces soman toxicity in mice. Harris *et al.* [21] also demonstrated that hemicholinium, a potent inhibitor of high-affinity choline uptake, protects against soman intoxication. Hopff *et al.* [22] found that methylmethanethiolsulfonate, another ChAT inhibitor, prolongs the time to death of lethal doses of both sarin and soman. Buccafusco *et al.* [23] reported that clonidine, an α_2-adrenergic receptor agonist, known to inhibit ACh release, protects against organophosphate toxicity.

We have demonstrated recently that *N*-hydroxyethyl-naphthylvinylpyridinium iodide, a quaternary ChAT inhibitor and *N*-allyl-3-quinuclidinol (NAQ), a potent high-affinity choline uptake inhibitor, administered in combination with atropine and pralidoxime, are both effective in reducing the percent mortality from soman over a 24-hr period in rats [18]. Carbamate *pretreatment* has been known to treat organophosphate intoxication effectively since 1946 when it was used against diiso-

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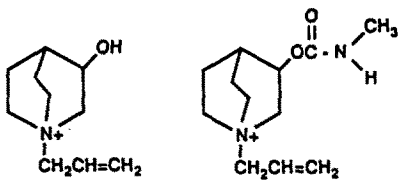
† Abbreviations: AChE, acetylcholinesterase; CAB, 3-carbamyl-*N*-allylquinuclidinium bromide; NAQ, *N*-allyl-3-quinuclidinol; ACh, acetylcholine; ChAT, choline acetyltransferase; and pChE, pseudocholinesterase.



NAQ CAB

Fig. 1. Comparison of the structures of *N*-allyl-3-quinuclidinol (NAQ) and 3-carbamyl-*N*-allylquinuclidinium bromide (CAB).

propylfluorophosphate [25]. More recently, physostigmine and pyridostigmine have been demonstrated to protect against the lethal effects of soman [26].

The present studies were designed to evaluate 3-carbamyl-*N*-allylquinuclidinium bromide (CAB), the carbamylated derivative of NAQ, for its pharmacological effects on cholinergic activity and potential protective effects against soman intoxication (see Fig. 1 for structures). The studies were based on the observed ability of NAQ to protect against soman and the reported efficacy of carbamates in the prevention of organophosphate toxicity. Reaction of CAB with acetylcholinesterase would lead to carbamylation of the enzyme and the release of NAQ as the leaving group, creating the possibility of enzyme protection and inhibition of high-affinity choline uptake with a single compound. Preliminary studies have been reported [27].

MATERIALS AND METHODS

Chemicals

CAB and NAQ were synthesized in these laboratories. Soman was obtained from the U.S. Army Institute for Chemical Defense. Atropine sulfate, pyridine-2-aldoxime methylchloride, assay enzymes and substrates were purchased from the Sigma Chemical Co. (St. Louis, MO). [^{14}C]-Acetylcholine (sp. act. 1–5 mCi/mmol) and [^{3}H]-choline (sp. act. 80–87 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Other chemicals were purchased from the Fisher Scientific Co. (King of Prussia, PA).

Chemical synthesis

To a solution of 3-quinuclidinol (1.0 g; 7.86 mM) in 10 mL of chloroform methylisocyanate (1 mL; 17 mM) was added with stirring, over a period of 10 min. The reaction mixture was then stirred at room temperature for 2 hr. The solvent and excess methylisocyanate were removed under vacuum. The compound was purified by column chromatography on alumina using chloroform as the eluent (R_f = 0.3) to give 1.2 g (83% yield) of a clear oil. The hydrochloride salt was prepared with a melting point of 174–176°. Quaternization of the carbamyl free base with an equimolar amount of allyl bromide in chloroform provided white crystals which were recrystallized from warm methanol. Separation of the optical isomers of 3-quinuclidinol was accomplished either by derivatization to acetoxyquinuclidine or by resolution of the alcohol itself using *p*-chlorotartranilic acid.

Cholinesterase assay

Erythrocyte AChE and plasma pseudocholinesterase (pChE) were assayed by the method of Siakotos *et al.* [28]. The assay mixture contained NaH_2PO_4 (20 mM), Na_2HPO_4 (80 mM), NaCl (300 mM), and polyoxyethylene ether (10.0 g/L) adjusted to pH 7.4. A 10% dilution of plasma or erythrocytes (0.1 mL) was added to the assay mixture (0.2 mL) with various concentrations of inhibitor and incubated at room temperature for 10 min. [^{14}C]Acetylcholine iodide (3 mM, final concentration) was then added and each sample incubated at 37° for 30 min. Non-enzymatic hydrolysis was corrected for by running parallel tubes, omitting the enzyme. The reaction was terminated by adding 5 mL of resin–dioxane slurry consisting of Amberlite RP-69 resin in dioxane (20 mg/100 mL). The volume of the sample was brought up to 10 mL with dioxane. The samples were lightly centrifuged (500 *g* for 5 min) to pack the resin; each supernatant was transferred to a scintillation vial containing 10 mL of modified Bray's scintillation fluid and counted in a liquid scintillation spectrometer.

$$\% \text{ Enzyme activity} = \frac{^{14}\text{C activity (in presence of inhibitor)}}{^{14}\text{C activity (enzyme control)}} \times 100$$

Estimate of decarbamylation

Initially, 750 μL of rat erythrocyte cholinesterase from saline-washed (5×) red cells was incubated with 250 μL of a 1 mM *R*-CAB solution at room temperature for 1 hr. Initial activity of the enzyme fell to 28%. Twenty microliters of this preparation was diluted 100-fold to reduce *R*-CAB concentration (0.5 μM) in the assay medium. Aliquots were incubated in pH 8.0 phosphate buffer, the reaction was stopped by adding acetylcholine, and the mixture was assayed for recovered activity as above.

The equation for the carbamylation reaction is:

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [EI]$$

$$[EI] \xrightarrow{k_3} E' \xrightarrow{k_4} E + \text{leaving groups}$$

where E = active enzyme, $[EI]$ = reversible enzyme–inhibitor complex, E' = carbamylated (inactive) enzyme, leaving groups = NAQ + MeOH + CO_2, $k_3' = k_3/K_i$, and k_4 = first order rate of decarbamylation.

High-affinity choline uptake

Male Sprague–Dawley rats (Charles River, Wilmington, DE), 180–200 *g*, were used in these experiments. Synaptosomes from cerebral cortex were obtained by the method of Cotman [29] as previously reported [30]. Animals were killed by decapitation and the cerebral cortex was collected

in ice-cold 0.32 M sucrose. Tissue was homogenized in 20 vol. of sucrose, and then centrifuged at 1300 *g* for 3 min. The supernatant was recentrifuged at 14,000 *g* for 20 min. The P2 pellet was resuspended in sucrose and further purified on a Ficoll gradient (4%, 6%, 13%) by centrifugation at 63,500 *g* for 60 min. The synaptosomal band (interface between the 6 and 13% layers) was diluted with 20 mL of sucrose solution and recentrifuged at 50,000 *g* for 20 min; the pellet was resuspended in Krebs–Ringer bicarbonate buffer (0.7 to 1 mg protein/mL) for analysis of choline uptake.

Synaptosomal uptake of choline was determined as described by Sterling *et al.* [31]. Synaptosomes were suspended in Krebs–Ringer bicarbonate buffer containing NaCl, 120 mM; KCl, 5 mM; $NaHCO_3$, 30 mM; $MgSO_4$, 1 mM; KH_2PO_4, 1 mM; $CaCl_2$, 3 mM; and D-glucose, 10 mM. To measure sodium-independent choline uptake synaptosomes were incubated in "low-sodium" (30 mM) buffer. The synaptosomal suspension was aerated for 5 min with 95%/5% O_2/CO_2 and preincubated for 5 min at 37°. [^{3}H]Choline (1 μCi) was then added and each sample was incubated for 4 min. Total choline concentration was 2 μM in studies evaluating potential uptake inhibitors. To measure inhibition, compounds to be tested were added just prior to the [^{3}H]choline.

Incubation was terminated by placing samples on ice and adding 1 mL of ice-cold buffer with 2 mM choline. Samples were filtered through presoaked Whatman GF/C glass microfiber filters and washed with buffer. Filters were placed in scintillation vials, Biofluor scintillation fluid was added and the samples were counted by liquid scintillation spectrometry. [^{3}H]Choline in buffer without synaptosomes was passed through filters to measure nonspecific binding. Protein was measured by the BioRad protein assay.

In vivo *protection studies*

Male Hartley guinea pigs (Ace Animals, Boyertown, NJ), 275–325 g, were used in all experiments evaluating protection. The animals were housed in a controlled environment facility with a 12 hr–12 hr light–dark cycle and were given laboratory chow and water *ad lib*. The LD_{50} for soman was determined using probit analysis and the dose checked on the morning of each experiment. The protocol of a typical study was as follows:

Group I. Guinea pigs received soman by subcutaneous injection at a starting dose of 50 μg/kg (LD_{90} dose in the presence of atropine sulfate). Atropine sulfate (16 mg/kg, i.m.) was administered 1 min post-soman.

Groups II, etc. The guinea pigs were treated with either CAB or NAQ, administered intramuscularly, 30 min prior to soman exposure. The doses chosen for *in vivo* protection studies were based on the potency of these compounds for inhibiting high-affinity choline uptake or cholinesterase, as determined by *in vitro* assay. The animals then received soman and atropine sulfate as in Group 1. The NAQ and CAB were further evaluated against increasing doses of soman, up to 5 LD_{50}s. Note that each potential protective compound was administered as a *single* dose prior to soman administration.

Each daily control and experimental group consisted of five animals. The drugs were evaluated on 3–5 separate experimental days. Following soman administration, animals were observed for a 24-hr period, during which behavioral manifestations and survival times were reported.

Significant differences in the percent mortality among drug treatments were established by paired *t*-tests.

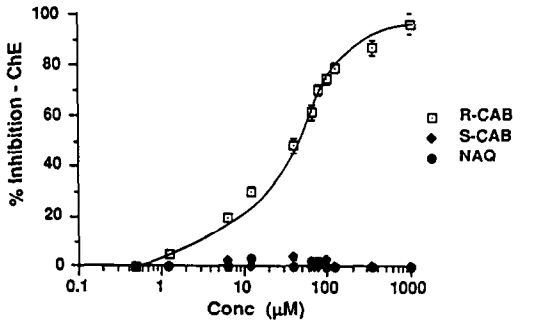


Fig. 2. Inhibition of erythrocyte cholinesterase activity by CAB and NAQ. A 10% dilution of red blood cells was incubated with stereoisomers of 3-carbamyl-*N*-allylquinuclidinium bromide to evaluate its effect on cholinesterase activity. Control ^{14}C recovered was 20,160 dpm. Each point represents the mean ± SEM of 4–6 samples.

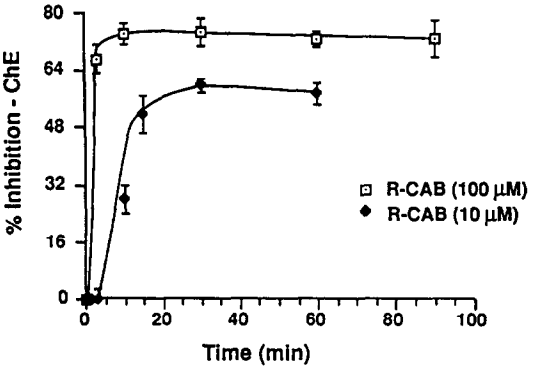


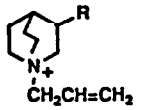
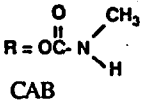
Fig. 3. Rate of carbamylation of erythrocyte cholinesterase by *R*-CAB (10 and 100 μM). Control ^{14}C recovered was 25,380 dpm. Each point is the mean ± SEM of 4 samples.

RESULTS

Cholinesterase activity

Since it is known that carbamates bind to and reversibly inhibit cholinesterase, the stereoisomers of CAB were evaluated for inhibition of erythrocyte and plasma cholinesterases. Inhibition of erythrocyte AChE is shown in Fig 2. The *R*-isomer demonstrated a concentration-dependent inhibition of erythrocyte enzyme activity (IC_{50} = 25 μM), whereas the *S*-isomer failed to inhibit at the same concentrations.

Table 1. Inhibition of high-affinity choline uptake

	Racemic		S-Isomer		R-Isomer	
	IC_{50}* (μM)	Max. % inh.†	IC_{50} (μM)	Max. % inh.	IC_{50} (μM)	Max. % inh.
R = OH NAQ	0.9‡	92 ± 3	0.1	94 ± 1	10.0	51 ± 3
 CAB	32.3	98 ± 2	63.0	98 ± 3	4.8	99 ± 1

* Concentration of compound (μM) producing 50% inhibition of high-affinity [^{3}H]choline (1 μCi, 2 μM) uptake. At least 8 concentrations of inhibitor from 10 nM to 100 μM were run (4–6 samples per concentration) to generate each IC_{50}.

† Maximum percent inhibition of high-affinity choline uptake at 100 μM. Values are means ± SEM.

‡ NAQ data were reported previously in Ref. 31.

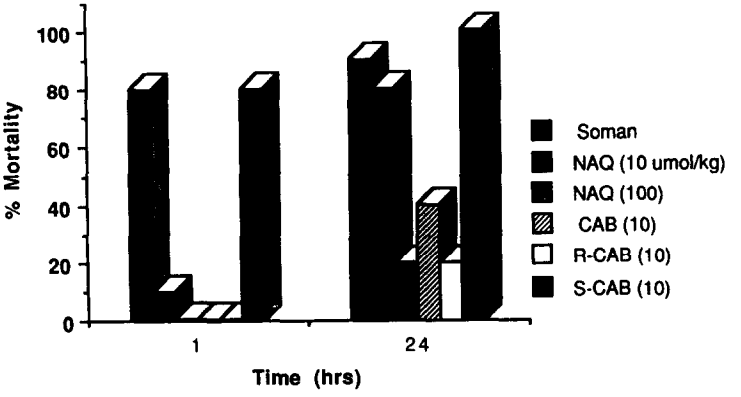


Fig. 4. Protection against soman toxicity by (a) NAQ and (b) CAB. NAQ and CAB were administered i.m., 30 min prior to soman. The soman was administered at 50 μg/kg, s.c., followed by atropine sulfate (16 mg/kg, i.m.) at 1 min post-soman.

The stereoisomers of CAB demonstrated similar results for the inhibition of plasma cholinesterase (IC_{50} for *R*-CAB = 29 μM). NAQ, the noncarbamylated quinuclidine derivative, failed to inhibit either AChE or pChE activity at concentrations up to 1 mM.

The rate of carbamylation, and hence inactivation of erythrocyte cholinesterase by *R*-CAB is shown in Fig. 3. Enzyme activity with 100 μM *R*-CAB was reduced with an inhibition half-life of 2.5 min and a maximum inhibition of 74.6%. At 10 μM, the half-life was 15 min with a ceiling of 60% inhibition.

The rate constants calculated from these data and the equation for carbamylation (see Materials and Methods) are: $k_3' = 550$ L/mol/min; $k_4 = 0.109$/min.

In a limited number of experiments, a high concentration of enzyme was first inhibited with *R*-CAB, centrifuged to remove excess CAB, and diluted 100-fold, and the return of enzyme activity followed at various time intervals. The k_4 rate of decarbamylation determined in this manner approximated the value calculated above and was similar to that reported for neostigmine by Dawson [32].

High-affinity choline uptake

NAQ has been shown to be a potent inhibitor of high-affinity choline uptake [31]. The carbamylated derivative CAB was therefore evaluated and compared to NAQ for its potential inhibitory effects (Table 1). Racemic CAB was a less potent inhibitor of high-affinity choline uptake than racemic NAQ. CAB demonstrated stereospecificity, with the *R*-isomer proving to be more potent for inhibition of high-affinity choline uptake than its corresponding *S*-isomer. Though this stereospecificity was consistent with its inhibition of cholinesterase, it differed from that of NAQ whose *S*-isomer was more potent for inhibition of high-affinity choline uptake than the *R*-isomer. Neither NAQ nor CAB significantly inhibited choline acetyltransferase.

Protection studies

NAQ and CAB were evaluated for their protective effects against soman intoxication in guinea pigs (Fig. 4). The LD_{50} of soman, administered subcutaneously, was 25 μg/kg. Soman, administered at 50 μg/kg, s.c., followed 1 min later by atropine sulfate (16 mg/kg, i.m.), produced 80% mortality in 1 hr and 90–100% mortality in 24 hr. NAQ (1–100 μmol/kg), administered 30 min prior to soman by intramuscular injection, markedly delayed the time to death from soman; it was most effective at 100 μmol/kg. When evaluated against the same dose of soman, racemic CAB (10 μmol/kg) also markedly reduced the percent mortality, at doses lower than NAQ.

Based on *in vitro* results, it was decided to evaluate the protective effects of the stereoisomers of CAB. The *R*-isomer of CAB (10 μmol/kg) markedly reduced the percent mortality, with no deaths observed in the first several hours. In contrast, the *S*-isomer of CAB (10–100 μmol/kg) was ineffective in protecting against soman. These data corresponded to the stereoisomeric differences of the *in vitro* potency of CAB as an inhibitor of high-affinity choline uptake and cholinesterase activity.

Based on the effectiveness of *R*-CAB and NAQ against 50 μg/kg soman, the compounds were also evaluated for protection against increasing doses of soman, up to 125 μg/kg (5 LD_{50}s) (Fig. 5). Though NAQ reduced an LD_{90} dose of soman (50 μmol/kg) to an LD_{20}, in the presence of atropine, protective effects were diminished with increasing doses of soman. In contrast, the carbamylated derivative, *R*-CAB (10 μmol/kg), protected against soman-induced deaths even at doses up to 5 LD_{50}s and reduced the mortality at 24 hr from 100 to 60%. Pralidoxime, given in conjunction with atropine and CAB, failed to enhance the 24-hr protection offered by atropine and CAB alone against 5 LD_{50}s of soman.

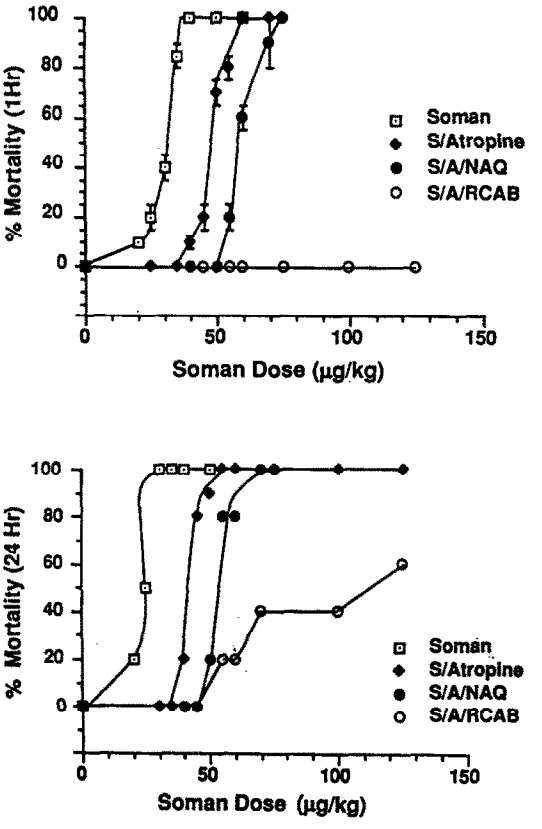


Fig. 5. Protection versus increasing doses of soman by NAQ and *R*-CAB administered at 100 and 10 μmol/kg, i.m., respectively, 30 min prior to soman. Atropine sulfate was administered 1 min post-soman. The percent mortality is presented at 1 hr (top panel) and 24 hr (bottom panel) post-soman. Five animals were used for each dose with experiments repeated on 3–5 separate experimental days (see Materials and Methods). Values are means ± SEM.

DISCUSSION

The present studies were performed to determine if a single compound, possessing the attributes of carbamates and inhibitors of ACh synthesis, would enhance the protection against soman toxicity afforded by atropine and other cholinolytics. CAB, synthesized based on this premise, is the carbamylated derivative of *N*-allyl-3-quinuclidinol, a potent inhibitor of high-affinity choline uptake *in vitro* [30, 31]. Furthermore, carbamylation of acetylcholinesterase with CAB yielded NAQ as the leaving group (Fig. 6). The results presented herein support the hypothesis that compounds which possess both attributes, e.g. CAB, demonstrate enhanced protective efficacy.

It is known that the addition of substrate levels of ACh (0.1 to 1 mM) protect the cholinesterases from further phosphorylation. The reaction of the enzyme with carbamates such as physostigmine is similar to the interaction with ACh. Initially, following complex formation, ACh acetylates cholinesterase, whereas physostigmine carbamylates the same site. Both reactions effectively prevent phosphorylation by organophosphates. As observed, *R*-CAB, but neither *S*-CAB nor NAQ, inhibited cholinesterase activity in a concentration–response manner. Thus, the protective efficacy of *R*-CAB in these experiments was in part attributed to its ability to carbamylate and reversibly inhibit cholinesterase. The overall rate of carbamylation observed was at least one order of magnitude slower than physostigmine, because of the nature of the leaving groups, NAQ from CAB versus eserinol from physostigmine [32]. The "phenolate" anion of eserinol is formed more easily than the corresponding alicyclic hydroxy anion of the quinuclidine compound and this would contribute to the overall carbamylation rate. The rate of enzyme decarbamylation was observed to be the same in both cases.

Ferguson *et al.* [33] have reported that *S*(+)-acetyl-β-methylcholine is a more potent inhibitor of high-affinity choline uptake than the corresponding *R*(−)-isomer. This is in agreement with our own previously reported observations [31] concerning the stereospecificity of inhibition exhibited by the isomers of NAQ. However, it was observed that the *R*(−)-isomer of CAB proved more potent an inhibitor of high-affinity choline uptake than its corresponding *S*(+)-isomer. Therefore, a molecular

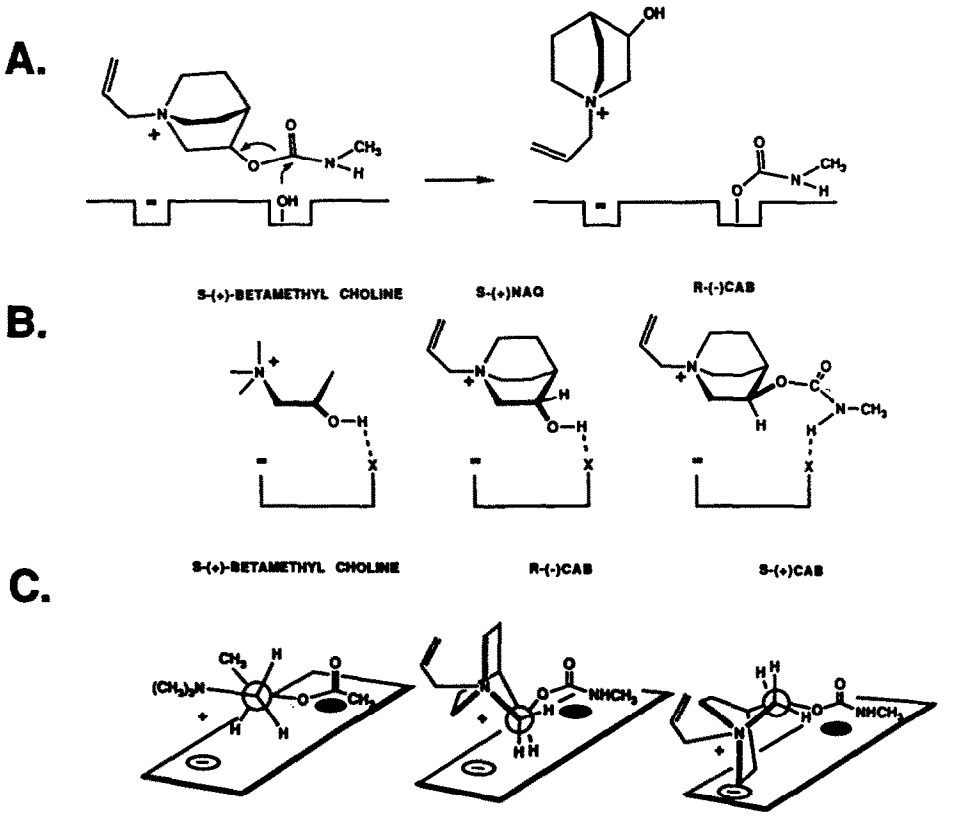


Fig. 6. Schematic of the interaction of CAB with cholinesterase. (A) CAB binds to AChE resulting in carbamylation of the enzyme and release of NAQ, an inhibitor of choline uptake, as the leaving group. (B) *S*(+)-Acetyl-β-methylcholine and *S*(+)-NAQ can achieve similar alignment with a hydrogen acceptor at a putative binding site; *R*(−)-CAB can more easily achieve this alignment than the corresponding *S*-isomer. (C) Newman projections depict the alignment of these compounds, suggesting that the appropriate interaction between substrate and enzyme in the case of *S*(+)-CAB is prevented by steric hindrance.

modelling program was employed to compare *S*-NAQ and *R*-CAB. The hydroxyl group of both *S*(+)-acetyl-β-methylcholine and *S*(+)-NAQ may act as a hydrogen donor in a hydrogen bonding interaction with a site on the high-affinity choline uptake system. Holding all other molecular coordinates constant, calculations indicate that the carbamate-hydrogen of *R*-CAB when compared to *S*-CAB can more easily occupy the same coordinates in space as that of *S*(+)-NAQ, and thus similarly contribute to H-bond formation (Fig. 6B). This may explain the reversal of potencies as a function of absolute configuration in CAB versus NAQ and acetyl-β-methylcholine.

Stereoselectivity with regard to interaction between AChE and substrates (acetyl-β-methylcholine) or inhibitors (organophosphates) has been demonstrated previously [34, 35]. In the present study, *R*(−)-CAB proved to be the inhibitory isomer, resulting in carbamylation of the enzyme; *S*(+)-CAB had no effect. Hoskins and Trick [34] have reported that the *S*(+)-isomer, but not the *R*(−)-isomer of acetyl-β-methylcholine was hydrolyzed by AChE. This inversion of stereospecific preference with regard to absolute configuration prompted a molecular modelling analysis. As depicted in Fig. 6C, both *S*(+)-acetyl-β-methylcholine and *R*(−)-CAB permit access to the carbamate group from the designated plane of enzymatic attack; the methyl group of acetyl-β-methylcholine and the bulk of the quinuclidine ring face away from the plane. In the case of *S*(+)-CAB, rotation of the ring to properly align the quaternary nitrogen and carbamate groups with the plane places a quinuclidine bridge in steric collision with the plane, which may explain the lack of reactivity between AChE and *S*(+)-CAB.

The protection afforded by *R*-CAB against soman lethality in guinea pigs corresponded to its *in vitro* potency as an inhibitor of high-affinity choline uptake and cholinesterase activity. This is in agreement with previous studies in rats in our laboratory and others, demonstrating that compounds which inhibit *in vitro* synthesis of ACh protect against soman toxicity *in vivo* [18, 20]. It has been suggested that the guinea pig provides a better animal model for predicting the efficacy of therapeutic regimens for organophosphate intoxication due to the better correlation with primates [26].

Though NAQ protects against soman in rats [30] and guinea pigs, the effectiveness of NAQ is rapidly diminished with increasing doses of soman. Carbamylation of NAQ to form CAB allowed for continued protection at all doses of soman evaluated, up to 5 LD_{50}s (125 μg/kg). This is in agreement with previous reports concerning physostigmine and pyridostigmine protection against organophosphates [9–13]. The fact that NAQ protects initially and also that its carbamylation to CAB enhances this protection indicate a role for both prevention of enzyme phosphorylation and inhibition of ACh synthesis. *R*-CAB thus represents a multipotent antagonist with regard to ACh synthesis and hydrolysis, its reaction with AChE yielding *R*(−)-NAQ, an inhibitor of high-affinity choline uptake albeit the weaker isomer of the quaternized alcohol.

Addition of pralidoxime to the regimen of CAB and atropine failed to enhance its protective effects. However, addition of a more lipid-soluble cholinesterase reactivator, with a longer duration of action, may increase the overall survival rate. It is important to note that the protection in this study was afforded through a single administration of drug. In therapy, continued periodic administration may produce even greater protection since the observed delayed deaths may have been due to release of additional amounts of soman from depot stores. The concept of depot forms is well documented in studies of this phenomenon by Clement [36] and by Wolthius *et al.* [37].

In summary, 3-carbamyl-*N*-allylquinuclidinium bromide protected against multiple LD_{50} doses of soman. This protection as well as its inhibition of high-affinity choline uptake and cholinesterase activity illustrated the importance of stereoselectivity. The results suggest that combining inhibition of ACh synthesis with reversible inhibition of cholinesterase enhances the protection afforded by cholinolytic agents.

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