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AFFINITY LIGANDS AND RELATED AGENTS FOR BRAIN MUSCARINIC AND NICOTINIC CHOLINERGIC RECEPTORS

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Abstract—This study describes the chemical synthesis and receptor binding characteristics of various affinity ligands and related ligands for brain muscarinic and nicotinic cholinergic receptors, including the 4-bromoacetamidobenzoic acid esters of dimethylaminoethanol (DMBAB) and choline (BABC) and 4-iodoacetamidobenzoylcholine (IABC). The reversible binding of [^{3}H]3-quinuclidinylbenzilate ([^{3}H]QNB) to calf brain membranes was inhibited in a concentration-dependent and saturable manner by DMBAB, BABC, and IABC with K_i values of 8×10^{-7}, 3×10^{-7} and 8×10^{-7} M, respectively; the K_i values for inhibition of reversible binding of the nicotinic ligand, [^{3}H]methylcarbamylcholine ([^{3}H]-MCC), were 1×10^{-6}, 6×10^{-8}, and 1×10^{-6} M, respectively. The K_i values for irreversible inhibition of [^{3}H]QNB binding were 8×10^{-7}, 1×10^{-7}, and 2×10^{-7} M for DMBAB, BABC, and IABC, respectively, and for [^{3}H]MCC binding, 8×10^{-5}, 1×10^{-5}, and 2×10^{-5} M, respectively. Although DMBAB was found to inhibit the QNB-induced hyperactivity in mice, it did not antagonize the toxic or other pharmacologic effects of oxotremorine. Structure–activity studies with various non-affinity analogues of the 4-aminobenzoate ester of dimethylaminoethanol and choline revealed that removal of the NH_2 moiety from the phenyl group increased affinity for the muscarinic but not the nicotinic cholinergic site, and quaternization of the ester side chain greatly increased affinity for the muscarinic site. Dimethylation of NH_2 in 4-aminobenzoylcholine decreased the affinity for both cholinergic sites. Replacement of NH_2 by NO_2 increased affinity for the muscarinic but not the nicotinic site, whereas quaternization of the 4-nitrobenzoyl ester markedly increased affinity for the nicotinic site while diminishing affinity for the muscarinic site. The findings indicate that DMBAB and its analogues are useful affinity ligands for examining the biochemical and functional characteristics of brain cholinergic receptors, particularly the muscarinic which has an affinity near the nanomolar concentration range.

In the course of investigating various agonists and antagonists to nicotine and their action at nicotinic receptors, it was noted that procaine and other local anesthetics, when administered intraventricularly, are antagonistic to the actions of nicotine [1]. Although it was surmised that the action of the local anesthetics was due primarily to their ability to produce nerve blockade, it was noted that procaine in micromolar concentrations also inhibits [^{3}H]-nicotine binding to rat brain membranes. Furthermore, it was found that procaine and cocaine both inhibit the binding of [^{3}H]mecamylamine [2], a ligand for ion channels associated with the nicotinic cholinergic receptor. Local anesthetics have been shown to inhibit nicotinic cholinergic function by acting at components distinct from those associated with nicotinic recognition and the regulation of voltage-gated cation conductance [3–5]. It also has been shown that procaine binds to the recognition site of M_2 muscarinic cholinergic receptors in guinea pig cecum [6].

One approach to investigating the sites of action of neuroreceptor ligands is the use of affinity ligands for both the receptor recognition and transducer sites [7–9]. A previous study [10] described the synthesis as well as the receptor binding characteristics and pharmacologic properties of a procaine-derived affinity ligand, 4-bromoacetamidoprocaine (BAP†). BAP was demonstrated to inhibit irreversibly both muscarinic and nicotinic cholinergic sites, the inhibition for the muscarinic site being two orders of magnitude greater than that for the nicotinic site. The primary aim of the present study was to synthesize BAP analogues that could be readily radiolabeled with [^{3}H]CH_3I and used to isolate and characterize the specific binding sites of the affinity ligand.

The present study describes the synthesis, receptor binding properties, and *in vivo* pharmacologic

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† Abbreviations: BAP, 4-bromoacetamidoprocaine; DMANB, dimethylaminoethyl-4-nitrobenzoate; DMAB, dimethylaminoethyl-4-aminobenzoate; DMBAB, dimethylaminoethyl-4-bromoacetamidobenzoate; DMABDM, 4-dimethylaminobenzoic acid ester of dimethylaminoethanolamine; DMBC, 4-dimethylaminobenzoylcholine·iodide; NBC, 4-nitrobenzoylcholine·iodine; ABC, 4-aminobenzoylcholine·iodide; BABC, 4-bromoacetamidobenzoylcholine·iodide; IABC, 4-iodoacetamidobenzoylcholine·iodide; MCC, methylcarbamylcholine; and QNB, 3-quinuclidinylbenzilate.

data of various substituted benzoate esters of alkylaminoalkyl alcohols, including the 4-bromoacetamidobenzoic acid ester of dimethylaminoethanol (DMBAB) and related affinity ligands.

METHODS

Synthesis of dimethylaminoethyl-4-nitrobenzoate (DMANB). To a flask containing 0.08 mol of 4-nitrobenzoylchloride was slowly added 0.10 mol of dimethylaminoethanolamine. After the mixture was refluxed for 2 hr under reduced pressure (50 mm), the contents were dissolved in 50 mL methanol, and the residue obtained after concentration was filtered and crystallized from a mixture of methanol and 2-propanol. The product after recrystallization from ethyl ether was obtained in 70% yield; m.p. of 143°. R_f values from silica gel TLC were 0.62 in 80% methanol and 0.79 in 1:1 ethyl acetate–methanol. Analytic: i.r. 1740, 1545, 1480, 1465, 1350, 1225. FABMS: molecular ion at m/z 250 (M + H; 100).

Synthesis of dimethylaminoethyl-4-aminobenzoate·HCl (DMAB). A 0.02-mol sample of DMANB, dissolved in a mixture of equal parts of methanol and ethanol, was subjected to hydrogenation for 2 hr with palladium black as a catalyst. After filtration through celite, the filtrate was concentrated *in vacuo*. Crystallization was performed from ethyl ether; m.p. 65°. R_f values in silica gel TLC were 0.75 in 80% methanol and 0.30 in 9:1 chloroform–methanol. Analytic: i.r. 1705, 1630, 1615, 1525, 1185, 1125. FABMS: molecular ion at m/z 209 (M + H; 5.31), m/z 164, m/z 120, glycerol adduct of m/z 164 at 256. NMR (D_2O) δ 7.87 (d, 2H), 6.64 (d, 2H), 4.41 (t, 2H), 2.77 (t, 2H), 2.39 (s, 6H), and 2.68 (broad s, 2H).

Synthesis of dimethylaminoethyl-4-bromoacetamidobenzoate · HBr (DMBAB). To a solution of 0.01 mol of DMAB dissolved in 50 mL of methylene chloride was slowly added with stirring at room temperature 0.015 mol of bromoacetylbromide, and the reaction was allowed to proceed for 10 min. After decantation of the solvent, the slightly yellow residue was dissolved in methanol, and the product obtained as crystals from ethyl acetate had a m.p. of 145°. R_f values from silica gel TLC were 0.75 in 80% methanol and 0.1 in 8:1 chloroform–methanol. NMR (D_2O), 2.9 (s, 6H), 3.5 (t, 2H), 4.5 (t, 2H), 7.5 (d, 2H), 7.9 (d, 2H) and 7.15 (d, 1H). FABMS: molecular ion at m/z 329.

Synthesis of 4-dimethylaminobenzoic acid ester of dimethylaminoethanolamine (DMABDM). To a solution of 0.02 mol of dimethylaminoethanolamine + 0.03 mol of triethylamine in 100 mL of toluene was added slowly with stirring at room temperature 0.025 mol of 4-dimethylaminobenzoyl chloride. The mixture was filtered to remove the triethylamine HCl, the filtrate was dried *in vacuo*, and the final product was crystallized from a mixture of petroleum ether and isopropanol, m.p. 150°. R_f values from silica gel TLC were 0.72 in 80% methanol and 0.68 in 1:1 ethyl acetate–methanol.

Synthesis of 4-dimethylaminobenzoylcholine·iodide (DMBC). DMABDM in methylene chloride was treated with an excess of iodomethane, and the residue was washed with and recrystallized from acetone, m.p. 248°. Silica gel TLC yielded R_f values of 0.53 in 80% methanol and 0.17 in 8:1 chloroform–methanol. NMR, 7.76 (δ, 2H), 6.66 (δ, 2H), 4.60 (m, 2H), 3.69 (m, 2H), 3.16 (s, 9H), and 4.88 (broad s, 2H). FABMS: quasimolecular ion at m/z 252 (M + H; 70.56) and m/z 193 (MH-N$(CH_3)_3$; 33.68).

Synthesis of 4-nitrobenzoylcholine·iodide (NBC). To a solution of 0.005 mol of DMANB in 40 mL methylene chloride at room temperature was added 0.01 mol of iodomethane and the reaction allowed to proceed for 30 min. The final product was filtered and washed with methylene chloride followed by acetone and dried, m.p. 220°. R_f values from silica gel TLC were 0.62 in 50% methanol and 0.79 in 1:1 ethyl acetate–methanol. NMR, δ 2.85 (s, 9H), 3.5 (t, 2H), 4.4 (t, 2H), 7.8 (d, 2H), and 7.95 (d, 2H). FABMS: molecular ion at m/z 254 (M + H; 39.26) and m/z 195 (MH-N$(CH_3)_3$; 7.18).

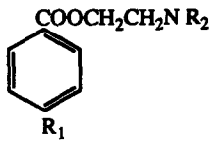
Synthesis of 4-aminobenzoylcholine·iodide (ABC). To a stirred solution of 0.005 mol of NBC in 2 mL of 6 N HCl was added in small portions zinc dust until the solution was almost colorless. After the solution was made alkaline with NaOH, the product was extracted with 50 mL acetone and dried *in vacuo*. Crystallization was performed in a mixture of isopropanol and ethyl acetate, m.p. = 90°. R_f values in silica gel TLC were 0.70 in 80% methanol and 0.55 in 1:1 ethyl acetate–methanol. FABMS: molecular ion at m/z 224 (M + H; 41.42) and m/z 165.

Synthesis of 4-bromoacetamidobenzoylcholine·iodide (BABC). To a solution of 0.01 mol of ABC in 2 mL dimethylformamide + an excess of Na_2CO_3 with stirring was slowly added 0.02 mol of bromoacetylbromide. The residue formed upon the addition of 40 mL methylene chloride was dissolved in a minimal amount of methanol and recrystallized from ethyl acetate, m.p. 125° decomp. R_f values from silica gel TLC were 0.34 in 80% methanol and 0.29 in 8:1 chloroform–methanol. FABMS: m/z 344 and m/z 346.

Synthesis of 4-iodoacetamidobenzoylcholine·iodide (IABC). To a solution of 0.005 mol of DMBAB in 1.0 mL of dimethylformamide was added 0.01 mol of iodomethane and the reaction allowed to proceed at room temperature for 2 hr. The amorphous residue that formed upon the addition of ethyl acetate was crystallized from ethyl acetate–isopropanol to yield IABC, m.p. 120°. FABMS: m/z 378.

Measurement of [^{3}H]methylcarbamylcholine ([^{3}H]-MCC) and [^{3}H]3-quinuclidinylbenzilate ([^{3}H]QNB) binding. The procedure for the measurement of reversible specific [^{3}H]MCC (a specific ligand for the nicotine cholinergic receptor) and [^{3}H]QNB binding has been described elsewhere [2, 11]. Membranes were obtained from cortical gray matter of fresh calf brains after homogenization in 0.05 M sodium phosphate buffer, pH 7.0, and centrifugation at 50,000 *g* for 30 min. The pellet was then resuspended in sodium phosphate buffer and stored on ice for up to 3 days. To a 2-mL polypropylene centrifuge tube was added 0.8 mL of 1.25 mg/mL membrane protein along with 0.1 mL of 1×10^{-8} M [^{3}H]MCC (80 Ci/mmol), or [^{3}H]QNB (45 Ci/mmol) with or without 10^{-5} M unlabeled MCC or QNB, respectively, in a

Table 1. K_i values for various analogues on [^{3}H]QNB and [^{3}H]MCC binding



Agent	R_1	R_2	[^{3}H]QNB	[^{3}H]MCC
Procaine	$-NH_2$	$(C_2H_5)_2$	4×10^{-6}	1×10^{-5}
DMAB	$-NH_2$	$(CH_3)_2$	8×10^{-6}	2×10^{-6}
ABC	$-NH_2$	$(CH_3)_3$	4×10^{-6}	8×10^{-7}
DMANB	$-NO_2$	$(CH_3)_2$	8×10^{-7}	1×10^{-6}
NBC	$-NO_2$	$(CH_3)_3$	1×10^{-4}	1×10^{-8}
BAP	$BrCH_2CONH-$	$(C_2H_5)_2$	3×10^{-7}	1×10^{-5}
DMBAB	$BrCH_2CONH-$	$(CH_3)_2$	8×10^{-7}	1×10^{-6}
BABC	$BrCH_2CONH-$	$(CH_3)_3$	3×10^{-7}	6×10^{-8}
IABC	ICH_2CONH-	$(CH_3)_3$	8×10^{-7}	1×10^{-6}
DMABDM	$(CH_3)_2N-$	$(CH_3)_2$	1×10^{-4}	1×10^{-5}
DMBC	$(CH_3)_2N-$	$(CH_3)_3$	7×10^{-6}	5×10^{-8}
DMB*		$(CH_3)_2$	1×10^{-6}	1×10^{-5}
BC*		$(CH_3)_3$	8×10^{-8}	2×10^{-5}

* Data from Ref. 11.

final volume of 1.0 mL. After incubation of the tubes in an ice bath for 60 min, the samples were filtered *in vacuo* on GB/F glass filters and washed twice with 3 mL of ice-cold buffer; radioactivity of the filters was determined by liquid scintillation. All assays were performed in triplicate and the experiments were repeated at least twice. Data are expressed as specific binding. K_i values were determined from log probit plots.

Measurement of irreversible binding to affinity ligands. The procedure for measuring irreversible binding after exposure to the affinity ligands DMBAB and BABC was as follows: Membranes were first exposed to various concentrations of either agent for various time periods at 37°, washed three times by homogenizing (polytron) the membrane pellet in 30 mL of ice-cold 40 mM sodium phosphate buffer, and centrifuged at 20,000 *g*. ^{3}H-Ligand binding was then measured as described above. K_i values were determined from log probit plots.

Pharmacologic measurements. DMBAB was tested for its ability to antagonize the pharmacologic and behavioral effects of nicotine or oxotremorine in mice. Mice were injected with 25 or 50 mg/kg of DMBAB, i.p., followed 3 min later by a dose of 1.0 mg/kg of nicotine or 3 mg/kg of oxotremorine, i.p. Parameters observed included tremors, seizures, muscle weaknesses, prostration, respiration, and salivation (oxotremorine).

Locomoter and general activity was measured with a Digiscan animal activity monitor linked to an IBM PC. The parameters recorded were vertical and horizontal movements and stereotypy (rearing, head-bobbing, and head swaying while in fixed location), activity data being expressed as the number of light beam interruptions/10-min period of measurement. All measurements were conducted between 10:00 a.m. and 2:00 p.m. After the activity was measured for a period of 10 min following an injection of 5 mg/kg of QNB, i.p., mice received a dose of 25 mg/kg of DMBAB i.p., and activity was measured for an additional 10 min.

RESULTS

Reversible inhibition of [^{3}H]QNB and [^{3}H]MCC binding by affinity ligands DMBAB, BABC, IABC and BAP. The affinity ligands DMBAB, BABC, IABC, and BAP had K_i values for reversible [^{3}H]-QNB binding to rat brain membranes of 8×10^{-7}, 3×10^{-7}, 8×10^{-7}, and 3×10^{-7} M, respectively, and K_i values for [^{3}H]MCC binding of 1×10^{-6}, 6×10^{-8}, 1×10^{-6}, and 1×10^{-5} M, respectively (Table 1).

Irreversible inhibition of [^{3}H]QNB binding by various affinity ligands. Inhibition of [^{3}H]QNB binding was measured in rat brain membranes after exposure of the membranes to various concentrations of the various affinity ligands for 1 hr at 37°, followed by washing to remove the unbound DMBAB. The K_i values for irreversible inhibition were 8×10^{-7}, 1×10^{-7}, 2×10^{-7}, and 7×10^{-8} M for DMBAB, BABC, IABC, and BAP, respectively (Table 2). In control experiments under comparable conditions, DMAB was without effect.

Irreversible inhibition of [^{3}H]MCC binding by various affinity ligands. The K_i values for irreversible inhibition of [^{3}H]MCC binding were 8×10^{-5}, 1×10^{-5}, 2×10^{-5}, and 9×10^{-7} M for DMBAB, BABC, IABC, and BAP, respectively (Table 2). In control experiments under comparable conditions, DMAB was without effect.

Time course of irreversible inhibition of [^{3}H]QNB binding by DMBAB. A plot of the time course of inhibition of [^{3}H]QNB binding by 1×10^{-6} M DMBAB revealed that the degree of inhibition

Table 2. K_i values for irreversible binding of various affinity ligands to calf brain membranes

Ligand	K_i (M)	
	[^{3}H]QNB	[^{3}H]MCC
DMBAB	8×10^{-7}	8×10^{-5}
BABC	1×10^{-7}	1×10^{-5}
IABC	2×10^{-7}	2×10^{-5}
BAP	7×10^{-8}	9×10^{-7}

K_i values were determined by log probit plots.

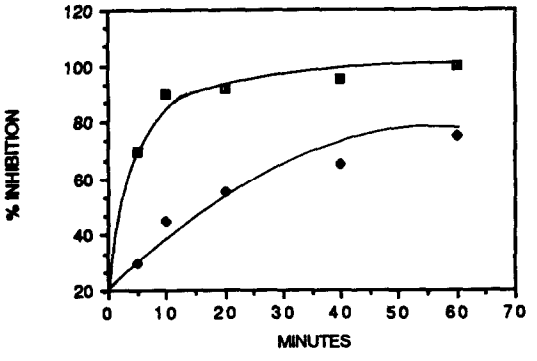


Fig. 1. Time course of irreversible inhibition of [^{3}H]QNB binding by DMBAB. Membranes were exposed for various times to 1×10^{-7} M (◆) or 1×10^{-6} M (□) DMBAB and washed prior to the measurement of [^{3}H]QNB binding. Control value for [^{3}H]QNB binding: 3.0 pmol/mg protein. The results are an average of two separate experiments run in triplicate; the variation was within 8% of the mean.

increased rapidly during the first few minutes, attained a near maximal value of 75% within 10 min, and remained constant throughout a period of 1 hr (Fig. 1). At a concentration of 1×10^{-7} M DMBAB, the degree of inhibition attained a maximum of about 60% and remained constant thereafter.

Effects of DMBAB, BABC, and IABC on [^{3}H]-*naloxone binding.* To determine the binding specificity of the ligands, control studies were performed with [^{3}H]naloxone. DMBAB, BABC, or IABC at concentrations of 1×10^{-4} M, which resulted in > 50% inhibition of [^{3}H]QNB and [^{3}H]-MCC binding, had no effect on reversible [^{3}H]-naloxone binding. When the membranes were exposed for 1 hr to the various affinity ligands and washed three times, no inhibition of [^{3}H]naloxone binding was observed.

K_i values for various analogues of DMAB on [^{3}H]-QNB and [^{3}H]MCC binding. Various compounds structurally related to DMAB and BAP were compared for inhibition of [^{3}H]MCC and [^{3}H]QNB binding (Table 1). Substitution of a dimethyl for the diethyl of procaine (DMAB) resulted in a slight decrease in the K_i for [^{3}H]QNB binding and a 5-fold increase in the affinity for [^{3}H]MCC binding. The addition of a third CH_3 at R_1 (ABC) increased the affinity for both sites still more, whereas the further addition at R_2 of dimethyl (DMBC) resulted in a decreased affinity for both binding sites. Replacement of NH_2 with NO_2 at R_1 in DMAB (DMANB) increased the affinity 10-fold at the [^{3}H]QNB site with little change at the [^{3}H]MCC site, while quaternization (NBC) diminished the affinity for [^{3}H]QNB 100-fold and increased that for [^{3}H]MCC 100-fold. As described elsewhere [11], one of the most potent nicotinic antagonists was benzoylcholine (BC) with a K_i of 8×10^{-8} M as compared with a K_i of 1×10^{-6} M for the tertiary analogue, dimethylaminoethyl benzoate, whereas both agents had a similar but lower affinity for the muscarinic site.

Table 3. Antagonism of QNB-induced hyperactivity by DMBAB and BAP in mice

	Activity (beam interruptions/10 min)		
	Horizontal	Vertical	Stereotypy
Control	2450 ± 655	295 ± 105	1601 ± 420
QNB	4150 ± 1235* (169)	520 ± 144* (176)	3485 ± 142* (217)
QNB + DMBAB	554 ± 452* (63)	238 ± 57 (81)	1358 ± 271* (85)
QNB + BAP	1758 ± 780* (72)	245 ± 62 (83)	1250 ± 190* (78)

Activity was determined for a period of 10 min after a dose of 5 mg/kg QNB i.p. Mice were then given 25 mg/kg of either DMBAB or BAP i.p., and activity was determined for an additional 10 min. Horizontal = locomotor activity; vertical = rearing activity; stereotypy = head bobbing, head swaying, and rearing while in fixed location in cage. Values are means ± SD, N = 6. Values in parentheses represent percent of control.

* P = 0.05 or less.

Reversal of QNB-induced increased in hyperactivity by DMBAB. DMBAB was tested for its ability to antagonize hyperactivity in mice (Table 3). At a dose of 5 mg/kg of QNB i.p., there occurred about a 69% increase in horizontal activity, a 76% increase in vertical activity, and a 117% increase in stereotypic behavior. When the mice were given a dose of 25 mg/kg of DMBAB, i.p. 20 min after the injection of QNB and tested 5 min later, horizontal activity was 63%, vertical activity 81%, and stereotypy 85% of the control value. In the absence of DMBAB, the hyperactivity persisted for at least 60 min. Comparable results were observed with BAP.

Antagonism of the effects of oxotremorine in mice by DMBAB and BAP. When mice were pretreated with 25 or 50 mg/kg of either DMBAB or BAP prior to the administration of oxotremorine, the toxic and other pharmacologic effects of oxotremorine, which included labored respiration, salivation, tremors, twitches, and prostration, were not altered significantly.

Effects of DMBAB and other agents on nicotine-induced seizures in mice. At doses of 25 or 50 mg/kg i.p., neither DMBAB nor BAP altered the incidence of seizures in mice following a dose of 2 mg/kg of nicotine hydrochloride, i.p. No antagonism was observed on other effects of nicotine, such as lower respiratory rate, decreased locomotor activity, and weakness in the hind limbs, as measured with a rotor rod. Other agents tested with negative effects were DMNB, NBC, DMABDM, and procaine at doses ranging from 10 to 50 mg/kg.

DISCUSSION

An aim of the present study was to develop additional affinity ligands for cholinergic receptors including one that could be readily ^{3}H-radiolabeled. In a previous study [10], it was shown that 4-bromoacetamidodiethylaminoethyl benzoate (BAP) was an affinity ligand for the brain muscarinic cholinergic receptor and, to a far lesser extent, the nicotinic cholinergic receptor. One shortcoming of BAP was that it could not be readily radiolabeled in the diethylaminoethyl side chain with [^{3}H]CH_3I as could the dimethylaminoethyl analogue of BAP. The present study has demonstrated that replacement of the diethyl moiety of BAP with dimethyl yielded DMBAB which had similar receptor binding affinities to BAP for both cholinergic receptors; the potency for the muscarinic receptor was two orders of magnitude greater than that for the nicotinic site. In addition to its irreversible inhibition of the muscarinic binding site, DMBAB was effective in antagonizing in mice the hyperactivity produced by the muscarinic antagonist QNB; however, it was without effect on the toxic or other pharmacologic effects of oxotremorine. In view of the fact that both affinity ligands blocked the action of QNB, the failure to observe blockade of the pharmacologic effects of oxotremorine, a muscarinic agonist, was unexpected. Since DMBAB is able to block the QNB-induced hyperactivity, it is able to enter the brain. The finding might be attributable to differences in the pharmacokinetic profiles of oxotremorine and DMBAB or to the possibility that some of the neurotoxic effects of oxotremorine are unrelated to its effects on muscarinic receptors [12]. Another possibility is that the muscarinic receptor subtype involved in the QNB-induced hyperactivity is distinct from that for oxotremorine. DMBAB appears to be devoid of any overt pharmacologic effects even at a dose of 50 mg/kg. Although procaine injected intraventricularly prevented the prostration and other effects produced by intraventricularly administered nicotine [13], neither procaine nor DMBAB administered systemically had any apparent effect on the behavioral effects of nicotine. It is likely that the brain level of procaine or DMBAB was below the 0.1 mM concentration required to inactivate open ionic channels [3, 4] or to saturate brain nicotinic recognition sites. Evidently, the effect of intraventricularly administered procaine in blocking the central effects of nicotine was via blockade of nerve conduction rather than through nicotinic recognition sites [13]. Although NBC exhibits a high affinity for the nicotinic receptor, it is evidently unable to penetrate the blood–brain barrier.

The rate of covalent interaction of DMBAB with brain membranes is very rapid, being complete within 10 min at a concentration of 1×10^{-6} M DMBAB. With the use of fast quenched-flowed techniques it has been shown that procaine inhibits the *Torpedo* nicotinic cholinergic receptor by acting at sites from nicotinic recognition sites responsible for triggering voltage-gated cation flux [3, 4]. The present study with rat brain membranes supports the notion that nicotinic agonists and procaine bind competitively at a common site. Insofar as both local anesthetics and nicotinic agonists also compete with [^{3}H]mecamylamine, a ligand for the cationic channel of the nicotinic cholinergic receptor [1, 14], it would appear that local anesthetics can interact with both agonist recognition and cationic channel sites. Independently of its action on muscarinic recognition sites, procaine has also been reported to be an inhibitor of calcium-induced release from the sarcoplasmic reticulum of calcium in various muscle preparations [15, 16].

Structure–activity studies with various analogues of the 4-aminobenzoate ester of dimethylaminoethanol (DMAB) revealed that removal of the NH_2 moiety from procaine increased affinity for the muscarinic but not the nicotinic cholinergic site, whereas quaternization of the ester side chain greatly increased affinity for the muscarinic site. Dimethylation of the 4-NH_2 in either DMAB or ABC markedly decreased the affinity for both cholinergic sites. Replacement of the NH_2 group of DMAB with highly electronegative NO_2 increased affinity for the muscarinic but not the nicotinic site, whereas quaternization of the 4-nitrobenzoyl ester significantly increased affinity for the nicotinic site while diminishing affinity for the muscarinic site. DMBAB and the other affinity ligands described should prove helpful in better characterizing the protein structures involved in various aspects of cholinergic receptor function, particularly the muscarinic receptor which appears to be considerably more sensitive to the ligands. They should also prove useful in pharmacologic and behavioral studies involving brain muscarinic receptors. Studies are

presently underway to utilize DMBAB for affinity chromatography to characterize the nature of the binding sites associated with the muscarinic receptors. Also underway are affinity-labeling studies with the use of [^{3}H]DMBAB.

Recently, another procaine affinity ligand, procaine isothiocyanate, has been demonstrated to be an irreversible inhibitor of synaptosomal binding of [^{3}H]batrachotoxinin-A, which is a specific ligand for voltage-sensitive sodium channels [17]. The affinity ligand inhibited binding in a noncompetitive, irreversible manner with an apparent K_i of 13 μM, whereas the inhibition by procaine was competitive and irreversible with an apparent K_i of 40 μM. No ligand binding studies on cholinergic recognition sites were reported.

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