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Nicotine antagonists: phosphoinositide turnover and receptor binding to determine muscarinic properties*

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A number of newly synthesized aminoalkyl and aminocycloalkyl esters of aromatic and substituted carbamic acids were demonstrated to be effective antagonists to the psychotropic and peripheral action of nicotine and other nicotinic agonists and to have a high affinity for the nicotine binding site in rat brain [1, 2]. The most potent nicotinic agonist of this series was methylcarbamylcholine (MCC), and [^{3}H]methylcarbamylcholine ([^{3}H]MCC) was found comparable to [^{3}H]nicotine in its binding characteristics and pharmacologic profile [2]. MCC is closely related to carbamylcholine (carbachol), which is a muscarinic agonist and readily binds to the muscarinic cholinergic receptor [3]. In light of these observations, a study was undertaken to investigate the effects of some of these compounds on the muscarinic cholinergic site.

It has been shown that activation of cholinergic muscarinic receptors in various tissues leads to increased hydrolysis of membrane phospholipids [4–7]. To determine whether some of the compounds which behaved as nicotinic antagonists also have some muscarinic effect, the compounds were evaluated for their abilities to inhibit carbachol-stimulated phosphatidylinositol (PI) turnover, and to compete for [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding.

Materials and methods

Chemicals. [^{3}H]Quinuclidinyl benzilate (44 Ci/mmol) and Aquasol-2 were obtained from New England Nuclear (Boston, MA), and *myo*-[2-^{3}H]inositol (20 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). AG-1-X8 (formate form) was purchased from Bio-Rad (Richmond, CA); carbamylcholine and DMAE benzoate were from Aldrich; and α-lobeline, atropine, and trihexyphenidyl were from the Sigma Chemical Co (St Louis, MO). *N*-Methyl-3-piperidyl benzoate, *N*-methyl-3-piperidyl benzoate methyl iodide, methylcarbamylcholine, 3-trimethylaminoethyl (TMAE) benzoate, quinuclidinyl benzoate, 2-dimethylaminoethyl (DMAE) phenylcarbamate, TMAE phenylcarbamate, DMAE cyclohexylcarboxylate, TMAE cyclohexylcarboxylate, DMAE phenylacetate, TMAE phenylacetate, DMAE phenylthiocarbamate and TMAE phenylthiocarbamate were synthesized as described elsewhere [1].

PI turnover and [^{3}H]*QNB binding.* Accumulation of [^{3}H]inositol phosphates in rat cerebral cortical slices was measured essentially according to the method of Berridge *et al.* [8] and Monsma *et al.* [9]. Inhibition of [^{3}H]QNB binding in rat brain membranes was investigated according to the method of Fisher *et al.* [10] and Monsma *et al.* [9]. The apparent K_i values for PI turnover and [^{3}H]QNB were calculated from the IC_{50} values according to the method of Cheng and Prusoff [11].

Results and discussion

The various compounds were compared for their abilities to inhibit carbachol-stimulated [^{3}H]IP accumulation in rat cerebral cortical slices and to inhibit [^{3}H]QNB binding in rat brain membranes (Table 1). The concentration of carbachol used in the PI turnover experiments was 1×10^{-4} M and the concentration of the radioligand in the binding assays was 1×10^{-9} M. The apparent K_i values obtained from the two types of experiments were comparable for *N*-methyl-3-piperidyl benzoate, *N*-methyl-3-piperidyl benzoate methyl iodide, quinuclidinyl benzoate, TMAE phenylcarbamate, DMAE and TMAE cyclohexylcarboxylate, DMAE and TMAE phenylacetate and TMAE phenylthiocarbamate. However, the K_i values obtained from the PI turnover experiments compared to the [^{3}H]QNB binding studies were nearly 5-fold greater for TMAE benzoate, 7-fold greater for DMAE benzoate and DMAE phenylthiocarbamate, 30-fold greater for DMAE phenylcarbamate, and 5-fold less for α-lobeline (Table 1).

Trihexyphenidyl, a muscarinic antagonist, was able to effectively inhibit in a dose-dependent manner the carbachol-stimulated PI turnover as well as the [^{3}H]QNB binding, the K_i values being 1×10^{-8} M and 1×10^{-9} M respectively. All the other compounds tested were found to be far less potent antagonists in their actions on the muscarinic receptor, the K_i values ranging between 10^{-6} and 10^{-4} M. Trihexyphenidyl had little effect on [^{3}H]nicotine or [^{3}H]MCC binding, the K_i values being greater than 10^{-4} M (Table 1).

Methylcarbamylcholine, TMAE phenylcarbamate, TMAE benzoate, quinuclidinyl benzoate and α-lobeline did not show any significant muscarinic agonist activity, even at a concentration of 10^{-4} M (data not shown). The stimulation of [^{3}H]IP accumulation above the basal was 7% for TMAE phenylcarbamate, 14% for MCC, 23% for TMAE benzoate, 26% quinuclidinyl benzoate and 43% for α-lobeline, whereas, for the muscarinic agonist carbachol the corresponding value was 412%. The stimulation caused by the various compounds was blocked by atropine (data not shown).

The present study demonstrates that some of the compounds which have been shown previously [1] to be nicotinic antagonists, of varying degrees of potency, also behaved as muscarinic antagonists, whereas some were pure nicotinic antagonists and had little or no effect on the muscarinic receptors. TMAE benzoate, TMAE cyclohexylcarboxylate, α-lobeline, TMAE phenylcarbamate, TMAE phenylacetate, TMAE phenylthiocarbamate have a much more profound effect on the nicotinic receptors than on the muscarinic receptors. Also, DMAE benzoate and DMAE cyclohexylcarboxylate affect the nicotinic receptors to a somewhat greater degree than the muscarinic receptors, whereas the actions of quinuclidinyl benzoate, DMAE phenylcarbamate, DMAE phenylacetate, DMAE phenylthiocarbamate and *N*-methyl-3-piperidyl-benzoate on both the nicotinic and muscarinic receptors are similar. It was found that among the various esters tested, except for TMAE and DMAE phenylacetate, the TMAE derivatives were much more effective than the DMAE derivatives, so far as the nicotinic receptors were concerned, both with respect to their binding affinity and pharmacologic action. TMAE and DMAE phenylacetate seemed to have similar effects on the nicotinic receptor. However, in the case of the muscarinic receptor, there was little difference in the potencies of the DMAE and TMAE derivatives.

The K_i value of trihexyphenidyl from [^{3}H]QNB binding was found to be 1×10^{-9} M, which agrees with the K_i value of 1.32×10^{-9} M obtained by Tien and Wallace [12]. The K_i value of trihexyphenidyl obtained from PI turnover

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Table 1. PI turnover, binding data, and pharmacology of various nicotine agonists and antagonists

Compound	K_i* (M) PI turnover	[^{3}H]QNB binding	[^{3}H]Nicotine binding	[^{3}H]MCC binding	Pharmacology† Brain ED_{50}‡ (nmol)	Heart ED_{50}§ (mg/kg)
N-Methyl-3-piperidyl benzoate	4×10^{-6}	4×10^{-6}	3×10^{-5}	2×10^{-5}	300	
N-Methyl-3-piperidyl benzoate methyl iodide	4×10^{-6}	4×10^{-6}				
α-Lobeline	3×10^{-6}	1.5×10^{-5}	5×10^{-9}	7×10^{-10}	10	0.2 ± 0.05
DMAE benzoate	1×10^{-4}	1.5×10^{-5}	1×10^{-6}		100	10.0 ± 25
TMAE benzoate	1×10^{-4}	2×10^{-5}	8×10^{-8}	4×10^{-8}	10	1.5 ± 0.3
Quinuclidinyl benzoate	4×10^{-7}	1×10^{-6}	7×10^{-6}	6×10^{-6}	200	5.0 ± 1.2
DMAE phenylcarbamate	1.5×10^{-4}	5×10^{-6}	1×10^{-4}	1×10^{-4}	IA	
TMAE phenylcarbamate	2×10^{-5}	2×10^{-5}	6×10^{-6}	8×10^{-7}	200	5.0 ± 1.0
DMAE cyclohexylcarboxylate	5×10^{-6}	1.5×10^{-5}	2×10^{-6}	4×10^{-6}	100	
TMAE cyclohexylcarboxylate	5×10^{-6}	2×10^{-5}	8×10^{-8}	5×10^{-8}	10	5.0 ± 1.5
DMAE phenylacetate	1×10^{-5}	4×10^{-5}	7×10^{-5}	7×10^{-6}	100	
TMAE phenylacetate	5×10^{-5}	$> 10^{-4}$	2×10^{-6}	3×10^{-7}	100	
DMAE phenylthiocarbamate	1×10^{-4}	1.5×10^{-5}	7×10^{-5}	2×10^{-5}		
TMAE phenylthiocarbamate	1.5×10^{-5}	4×10^{-5}	5×10^{-7}	5×10^{-7}	50	4.0 ± 0.8
Trihexyphenidyl	1×10^{-8}	1×10^{-9}	$> 10^{-4}$	$> 10^{-4}$	>500	

Abbreviations: DMAE, 2-dimethylaminoethyl; TMAE, 3-trimethylaminoethyl; and IA, inactive.
* K_i = apparent K_i.
† Pharmacologic data were obtained from Ref. 1.
‡ ED_{50} for blockade of nicotine-induced prostration.
§ ED_{50} for antagonism of hypertensive action of nicotine. Values are means ± SD, N = 5.

experiments was 1×10^{-8} M and is comparable with the value ($K_i = 2.5 \times 10^{-8}$ M) obtained by Monsma *et al.* [9] in rat striatum. Trihexyphenidyl is a potent muscarinic antagonist but, as revealed from [^{3}H]nicotine and [^{3}H]MCC binding assays, has little nicotinic action.

MCC is not capable of stimulating the breakdown of inositol phospholipids nor is it significantly effective in displacing [^{3}H]QNB binding; only at a concentration of 4×10^{-4} M can it inhibit 50% of the [^{3}H]QNB binding. It has been reported [13] that muscarinic drugs (oxotremorine and atropine) are poor competitors for the [^{3}H]MCC binding sites. On the other hand, MCC, like nicotine, has a profound psychotropic action, and is very potent in inhibiting [^{3}H]nicotine and [^{3}H]MCC binding, the K_i values being 8×10^{-9} M and 6×10^{-9} M respectively [2]. Araujo *et al.* [13] have shown that the effect of MCC to increase acetylcholine release from rat hippocampus and frontal cortex is antagonized by the nicotinic antagonists dihydro-β-erythroidine and *d*-tubocurarine but not by the muscarinic antagonist atropine. It is interesting that the N-methyl substituent in carbamylcholine increases the affinity for the nicotinic site, while virtually abolishing the affinity for the muscarinic cholinergic site.

In summary, a number of nicotinic antagonists and agonists, which were assayed previously for [^{3}H]nicotine binding, were evaluated for their muscarinic cholinergic activity, by determining their competition for [^{3}H]QNB binding to rat brain membranes and their effect on muscarinic receptor-mediated phosphatidylinositol (PI turnover). A number of potent nicotinic antagonists, such as trimethylaminoethyl esters of aromatic and cycloalkyl carboxylic acids, were less potent by over 4 orders of magnitude than the protolypic antagonist, trihexyphenidyl, in inhibiting carbachol-stimulated PI turnover. Quinuclidinyl benzoate and α-lobeline were both effective nicotinic antagonists and inhibitors of PI turnover. With the exception of quinuclidinyl benzoate and α-lobeline, most of the other nicotinic antagonists were relatively weak inhibitors of PI turnover, their action being attributable to their weak affinity for the muscarinic cholinergic recognition site.

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The changes in activities of some ammonia metabolizing enzymes in liver and brain of rats intoxicated by chronic administration of acetaldehyde

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Acetaldehyde (AcH) is a main natural metabolite of ethanol oxidation *in vivo*. Its ability to initiate hepatotoxicity has been well documented [1]. AcH is considered to be 10 to 30 times more toxic than ethanol [2]. It is capable of interacting with cellular constituents like proteins [3], glutathione [4] or many enzymes, and of modifying in this way metabolic functions of liver and other organs. AcH inhibits protein synthesis in the liver [5] and in the pancreas [6] as well as secretion of glycoproteins [7].

In rats treated with AcH for 4 weeks the increase of aspartate aminotransferase, alanine aminotransferase and γ-glutamylotranspeptidase activities was observed in the serum [8]. Histomorphological studies revealed a marked steatosis, necrosis of single cells and microthrombosis in the liver [9]. Some alterations caused by AcH have also been found in liver cell mitochondria resulting in impairment of citric acid cycle [10]. The changes in protein and nonprotein sulfhydryl compounds of rat plasma, liver and brain after chronic AcH administration has been also observed by several authors [4, 11, 12].

In rats chronically intoxicated with ethanol the increase of liver ammonia concentration and changes in the activities of ammonia metabolizing enzymes in the liver and brain were found [10, 13].

The aim of the present study was to examine the effect of chronic acetaldehyde administration on the activities of ammonia metabolizing enzymes in liver and brain, i.e. glutamine synthetase, glutamate dehydrogenase and glutaminase, and on the blood ammonia concentration in rats.

Materials and methods

Animals. The experiments were carried out on male Wistar rats weighing about 300 g. The animals were fed the standard commercial rodent diet *ad lib*. The rats were divided into two groups. The first group of 12 rats was given intragastrically 1.5% (w/v) solution of AcH in an isotonic NaCl in a daily dose of 0.3 ml/kg of body weight, every day for a period of 4 weeks. The second (control) group of 12 rats received an equivalent volume of 0.15 M NaCl. All rats were weighed before and after the experiment to determine the differences between mean initial and final body weight of each group. Mean daily food intake in AcH and control rats were compared and analysed by the *t*-test.

Preparation of tissues. After the blood had been taken, the liver and brain tissues were quickly removed and placed into 0.15 M NaCl in an ice bath. The tissues were subsequently blotted on filter paper in order to remove the physiological salt, then weighed and homogenized in 9 vol. of ice-cold 0.25 M sacharose. The homogenates were centrifuged at 600 *g* for 10 min at 4° and supernatant was kept in ice until assayed. Protein was determined in diluted aliquots of the tissue homogenates by the method of Lowry *et al.* [14], using crystalline bovine serum albumin as a standard.

The liver and brain glutamine synthetase activity was estimated according to the method of Rowe *et al.* [15]. The glutamate dehydrogenase activity was determined by the method of Schmidt [16], and glutaminase activity was measured by the method described by Mardashev *et al.* [17]. All enzyme activities were determined at 25°. One unit of enzymatic activity was defined as the amount of enzyme which catalysed the synthesis of μM of γ-glutamylhydroxamate, the amount of oxidized NAD and ammonia formed per mg of protein per hr for glutamine synthetase, glutamate dehydrogenase and glutaminase, respectively.

Blood ammonia concentration. Blood was taken by cardiac puncture into 2 ml heparinized tubes and analysed for ammonia concentration according to the method described by Hilgier and Albrecht [18].

Statistical analysis. Data were expressed as mean ± SD. The results were elaborated statistically using Student's *t*-test.

Results

No differences were found in final body weight between AcH and control rats groups. A marked increase of blood ammonia concentration in AcH treated group was observed, from 41.1 to 82.8 μmol/l. The differences were statistically significant ($P < 0.05$) (Table 1).

Table 2 shows the effect of AcH on the liver and brain glutamate dehydrogenase, glutamine synthetase and glutaminase activities in the rats. In AcH-treated groups the liver activities of glutamate dehydrogenase and glutaminase were higher than in the control group and the differences were statistically significant. However, the differences in the liver activity of glutamine synthetase were not significant ($P = 0.12978$). In the brain tissue of the AcH-treated group the activities of all analysed enzymes were significantly higher than in control group.

Discussion

It has been widely demonstrated that 4 weeks intoxication with AcH does not lead to the loss of body weight in the rats, while both studied organs—liver and brain—