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Pharmacological Evaluation of Methylcarbamylcholine-Induced Drinking Behavior in Rats

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YANG, X.-H., J. J. BUCCAFUSCO AND J. R. PAULY. *Pharmacological evaluation of methylcarbamylcholine-induced drinking behavior in rats.* PHARMACOL BIOCHEM BEHAV **49**(1) 1-6, 1994. —Methylcarbamylcholine (MCC), a structural analog of carbachol (an acetylcholine agonist), has been reported to be a specific nicotinic cholinergic receptor ligand. MCC produces a robust polydipsic response shortly following central administration. The purpose of the present study was to pharmacologically characterize this increase in drinking behavior. Male Wistar rats were implanted with intracerebroventricular (ICV) cannula guides directed at the left lateral ventricle. Following a recovery period, animals were injected ICV with saline or various doses of MCC (3-60 μg) and water consumption was quantified. MCC produced a dose-related, transient increase in water consumption that peaked at a dose of 30 μg. In contrast, nicotine, a potent nicotinic cholinergic receptor agonist, did not produce changes in drinking following ICV administration. MCC-induced increases in drinking were not blocked by pretreatment with several selective nicotinic receptor antagonists including dihydro-β-erythriodine (DHBE), hexamethonium, and mecamylamine. However, pretreatment with the muscarinic antagonist atropine (0.01 or 1.0 μg) completely abolished MCC-induced polydipsia. Following a chronic treatment regimen (MCC injected ICV twice daily for 10 days), no tolerance to MCC-induced changes in water consumption was observed. Previous studies have demonstrated that tolerance develops to nicotinic-receptor mediated responses following the identical chronic treatment paradigm. These results suggest that MCC-induced polydipsia is mediated through stimulation of muscarinic rather than nicotinic receptors.

Drinking behavior Polydipsia Methylcarbamylcholine Nicotine Mecamylamine Hexamethonium Dihydro-β-erythriodine Atropine Cholinergic

ACETYLCHOLINE (ACh) is a predominant neurotransmitter in the mammalian central nervous system. The effects of ACh on neuronal processes are mediated through both nicotinic and muscarinic cholinergic receptors. Over the past 10 years there has been a substantial increase in the study of brain nicotinic receptors, due largely to advances in the understanding of these proteins at the molecular level as well as the recognition that a selective loss of these receptors occurs in certain neurodegenerative disorders. There are at least three major subtypes of brain nicotinic receptors based on information obtained from ligand binding assays using L-[^{3}H]-nicotine, α-[^{125}I]-bungarotoxin, and [^{125}I]-neuronal bungarotoxin (also known as kappa toxin, toxin F and bungarotoxin 3.1) (35). The binding site identified by L-[^{3}H]-nicotine has received the most attention because it mediates many of the central actions of nicotine, and also because the number of these receptors is reduced (compared to age-matched controls) in postmortem tissue obtained from Alzheimer's patients [for review, see (29)]. However, there are various problems associated with using L-[^{3}H]-nicotine in ligand binding assays. For example, L-[^{3}H]-nicotine obtained from commercial sources contains contaminants that appear to be responsible for the identification of low affinity binding sites. Purification of L-[^{3}H]-nicotine by thin layer chromatography eliminates the appearance of these low affinity sites in binding assays, but as much as 40% of the isotope can be lost during the purification (34). Thus, other ligands that bind to the L-[^{3}H]-nicotine receptor have been developed.

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[^{3}H]-ACh (in the presence of atropine to block binding to muscarinic receptors) binds to the same site identified by L-[^{3}H]-nicotine (12,25). However, ACh is very sensitive to hydrolysis and, thus, has only limited usefulness in ligand binding assays. Two additional ligands have been developed that appear to label the L-[^{3}H]-nicotine binding site: [^{3}H]-cytisine (30) and [^{3}H]-methylcarbamylcholine (MCC; also known as methylcarbachol) (2). MCC is a methylated analog of the mixed muscarinic/nicotinic cholinergic agonist carbachol. Biochemical data suggest that [^{3}H]-MCC is a specific nicotinic receptor ligand. Classical binding assays (Scatchard plots, displacement by agonists and antagonists, etc.) have demonstrated that high affinity MCC binding to brain nicotinic cholinergic receptors is saturable and has virtually the same pharmacological profile as L-[^{3}H]-nicotine binding (2,9). Autoradiographic studies have shown that [^{3}H]-MCC binds to brain slices in a pattern that is comparable to L-[^{3}H]-nicotine and [^{3}H]-ACh (in the presence of atropine to block binding to muscarinic receptors) (9,41).

A variety of physiological and behavioral evidence also indicates that MCC is a specific nicotinic cholinergic ligand. MCC induces the release of [^{3}H]-ACh from hippocampal and cortical synaptosomes, and this effect is blocked by pretreatment with either dihydro-β-erythriodine or *d*-tubocurarine (nicotinic receptor blockers) but not by atropine (a potent muscarinic receptor blocker) (5). Boksa et al. (8) demonstrated MCC-induced stimulation of a number of nicotinic responses including depolarization of isolated rat sympathetic ganglia, release of [^{3}H]-norepinephrine from cultured adrenal medullary cells, contraction of frog rectus abdominus muscle, increases in blood pressure, and stimulation of striatal [^{3}H]-dopamine release. Each of these responses to MCC were mimiced by nicotine and blocked by dihydro-β-erythriodine (DHBE) or mecamylamine (a drug that blocks nicotinic receptor ion channels), but not by atropine. Marks et al. (23) demonstrated that MCC is a potent nicotinic agonist in an assay that measures rubidium efflux from synaptosomes as an indicator of the functional status of brain nicotinic receptors. Central injections of nicotine produce a behavioral prostration response (characterized by splaying of the limbs and immobilization) that was initially described by Abood et al. (1). MCC also produces a prostration response following intracerebroventricular (ICV) administration and this effect is blocked by mecamylamine (8) or DHBE (42) but not by scopolamine (a muscarinic antagonist) (8).

Studies that have examined the effects of chronic nicotine treatment also suggest that MCC is a specific nicotinic agonist. When rodents are chronically exposed to nicotine (or other nicotinic ligands such as cytisine, anabasine, and anatoxin) there is a paradoxical increase in the number of brain receptors labeled by L-[^{3}H]-nicotine or [^{3}H]-ACh [for a review, see (40)]. Postmortem analysis of human brain tissue indicates that tobacco users have increased numbers of brain nicotinic receptors compared with age-matched subjects who were not tobacco users (7). Lapchak et al. (18) demonstrated that chronic injections of nicotine increase the number of [^{3}H]-MCC binding sites in several rat brain regions. Furthermore, chronic ICV administration of MCC (twice daily for 10 days) increases the number of cortical nicotinic receptors identified by [^{3}H]-cytisine; pretreatment with DHBE significantly reduces MCC-induced increases in nicotinic receptor binding (42).

During the course of experiments that investigated the effects of chronic central MCC injection on brain nicotinic receptors, we observed that MCC injections consistently induced a robust polydipsic response. This effect of MCC was surprising because there is little evidence that nicotinic receptor ligands increase water consumption in rodents. Although Stein and Seifter (37) reported that central injections of nicotine produce a slight increase in water comsumption in monkeys, most studies have concluded that nicotine administration has an antidiuretic effect (which should reduce water consumption), possibly due to nicotine-induced increases in vasopressin secretion (17). On the other hand, many studies have reported that muscarinic cholinergic receptors are involved with drinking behavior. Several studies have demonstrated that central injections of the mixed nicotinic/muscarinic agent carbachol induces a polydipsic response (4,15,16, 19,26,27,33,36–39). Carbachol-induced increases in drinking behavior can be blocked by either atropine (39) or by M_1 selective muscarinic antagonists (33). Central injections of ACh (11,20), as well as the acetylcholinesterase inhibitor diisoproprylfluorophosphate (DFP) (14), have also been shown to stimulate water consumption in rodents. DFP-stimulated polydipsia can be blocked by either atropine or the M_1 selective antagonist pirenzepine but not by mecamylamine (14). Thus, the involvement of brain muscarinic systems in the initiation of drinking behavior is fairly convincing. The purpose of the present study was to thoroughly evaluate the pharmacological basis of drinking behavior induced by central administration of MCC.

METHOD

Materials

Male Wistar rats (Harlan Sprague–Dawley, Indianapolis, IN) were housed in separate cages and maintained on a 12L : 12D cycle (lights on at 0600 h) with unlimited access to food and water. The sipper tube of each water bottle was fitted with a stainless steel ball that prevented excess water leakage. Brevital sodium (Methohexital sodium) was purchased from Eli Lilly Pharmaceutical Co. (Indianapolis, IN). Cannula guides, cranioplastic powder, cranioplastic liquid, and other stereotaxic surgical supplies were purchased from Plastics One Inc. (Roanoke, VA). Nicotine bitartrate, hexamethonium, mecamylamine, and atropine were purchased from Sigma Chemical Co. (St. Louis, MO). Methylcarbamylcholine chloride and dihydro-β-erythriodine (DHBE) were generously supplied by Dr. Leo G. Abood (Department of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, NY) and Merck Sharp & Dohme Research Lab, a division of Merck & Co., Inc. (Rahway, NJ), respectively.

Surgery

Rats (300–400 g) were anesthetized with intraperitoneal injections of Brevital sodium (60 mg/kg) and placed in a Kopf (Tujnuga, CA) stereotaxic instrument. A small hole was drilled in the skull 0.4 mm posterior and 2.5 mm lateral to bregma [coordinates taken from (31)]. A 23 gauge stainless steel cannula guide was then implanted 3 mm below the dorsal surface of the skull, fixed at the base with acrylic cement, and anchored to a small screw. The cannula guide was protected by screw on cap and incisions were closed with surgical stitches. The placement of the cannula was verified by withdrawing 2 μl of cerebrospinal fluid through an injection cannula 5 days following surgery. ICV injections were performed with a 30 gauge cannula that was inserted through the cannula guide to a depth of 4.5 mm below the dorsal skull surface.

Drug Treatment

Rats were randomly assigned to various drug treatment groups. All drugs were dissolved in sterile saline. Injection volumes of 5 or 10 μl were accomplished over 15 or 30 s, respectively. In the initial experiments, rats were injected with various doses of MCC (3, 15, 30, or 60 μg in 10 μl of saline) or nicotine bitartrate (5, 12.5, 25, 50, or 100 μg) to obtain dose-effect relationships. These experiments were performed between 0930 and 1100 h. The time between the cessation of the injection and the onset of water consumption (drinking latency) was measured. The total volume of water consumed in 1 h following each injection was then determined. Animals were weighed prior to each injection so that data could be expressed in terms of ml water consumption per kilogram of body weight. In some experiments, animals were pretreated with saline or various different muscarinic and nicotinic cholinergic receptor antagonists including: DHBE (competes with MCC for high affinity binding to nicotinic receptor alpha subunits), mecamylamine (a nicotinic receptor channel blocker), hexamethonium (a competitive nicotinic receptor blocker), and atropine (a potent but nonselective muscarinic receptor antagonist). These drugs were dissolved in 5 μl of saline and injected 15 min prior to MCC (30 μg) challenge. In these experiments, water consumption was monitored for 1 h following the second injection. In some experiments, animals were treated chronically with MCC (30 μg, ICV twice daily for 10 days). Water consumption following each injection was determined for 1 h; overnight water consumption (from 1 h after the second daily injection to 0800 the next morning) was also determined in these animals. Chronic injections were delivered between 0900 and 1700 h on each day, with a 6-h interval between treatments.

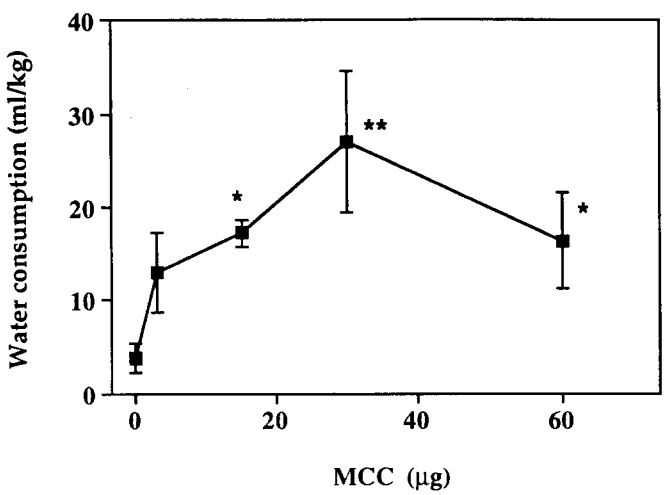

FIG. 1. Dose-effect relationship for drinking behavior induced by central injections of saline (n = 7) or MCC (3 μg, n = 5; 15 μg, n = 3, 30 μg, n = 21; and 60 μg, n = 4). Water consumption was recorded for 1 h following ICV injection of MCC and is expressed as ml per kg of body weight. Data represent mean ± SEM. Central injections of MCC produced a dose-related increase in water consumption, $F(4, 35) = 3.46$, $p < 0.05$, ANOVA. *$p < 0.05$, **$p < 0.01$ compared to saline control. Water consumption following the 30 μg dose of MCC was significantly higher than following other MCC doses ($p < 0.05$).

TABLE 1

THE EFFECTS OF VARIOUS NICOTINIC AND MUSCARINIC LIGANDS ON WATER CONSUMPTION INDUCED BY ICV ADMINISTRATION OF MCC

Treatment	Water Consumption (ml/kg)	n
Saline	3.83 ± 1.59	7
Nicotinic Agonists		
MCC 30 μg	21.99 ± 3.18*	11
Nicotine 5 μg	2.00 ± 1.0	3
Nicotine 12.5 μg	1.65 ± 0.65	3
Nicotine 25 μg	1.72 ± 0.57	3
Nicotine 50 μg	5.29 ± 2.0	3
Nicotine 100 μg	4.03 ± 2.54	3
Pretreatment With Antagonists		
DHBE 60 μg	2.77 ± 1.03	7
DHBE 60 μg + MCC 30 μg	22.55 ± 5.00*	6
Mecamylamine 30 μg	4.36 ± 1.75	3
Mecamylamine 30 μg + MCC 30 μg	25.88 ± 6.2*	4
Hexamethonium 50 μg	2.45 ± 1.34	3
Hexamethonium 50 μg + MCC 30 μg	24.30 ± 7.00*	5
Atropine 0.01 μg	4.80 ± 1.6	3
Atropine 0.01 μg + MCC 30 μg	4.09 ± 0.86†	8
Atropine 1 μg	7.87 ± 1.54	3
Atropine 1 μg + MCC 30 μg	4.08 ± 0.86†	8

Each value represents the mean ± SEM.
*$p < 0.05$, compared to control.
†$p < 0.05$ compared to MCC, Fisher's PLSD post hoc test.

Data Analysis

Data derived from each acute treatment group were analyzed using one-way analysis of variance (ANOVA) with Student's t-test or Fisher's protected least significant difference (PLSD) as post hoc tests. Chronic MCC treatment data were analyzed by one-way ANOVA with repeated measures.

RESULTS

Central injections of MCC produced a dose-related increase in water consumption, $F(4, 35) = 3.458$, $p < 0.05$, ANOVA (Fig. 1). Maximal stimulation of water consumption was measured following a dose of 30 μg of MCC ($p < 0.01$). MCC-induced polydipsia was transient because the control and drug-treated animals did not differ in overnight water consumption (data not shown). The onset of the drinking response was inversely related to the dose of MCC administered (i.e., higher MCC doses were associated with longer latencies for the initiation of drinking) (data not shown). The inverse relationship between latency to initiate water consumption and MCC dose is due to the extended duration of the prostration syndrome observed following treatment with higher doses of MCC.

Various cholinergic agonists and antagonists were used to evaluate the pharmacological basis of MCC-induced polydipsia. No changes in drinking behavior were observed following ICV injections of nicotine bitartrate (5, 12.5, 25, 50, or 100 μg) (Table 1). The failure of nicotine to induce an increase in water consumption suggested that nicotinic receptor stimulation does not mediate the effects of MCC. This possibility was supported by studies with the nicotinic receptor antagonists DHBE, hexamethonium, and mecamylamine. When these

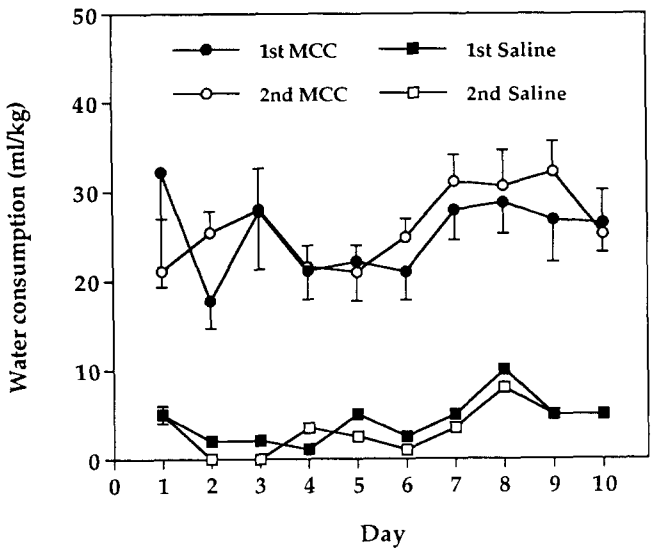

FIG. 2. The effects of chronic administration of MCC on water consumption. MCC (30 μg) was injected ICV twice daily for 10 days and water consumption was measured in the following hour. No significant tolerance developed to the effects of MCC (n = 6 for each treatment). For some points the error bars were smaller than the diameter of the symbol.

compounds were administered alone, no changes in drinking behavior were observed. MCC-induced polydipsia was not inhibited by the pretreatment (15 min before MCC injections) with these drugs (Table 1). However, pretreatment with atropine (0.01 or 1.0 μg), a muscarinic receptor antagonist, completely abolished the MCC-induced drinking, $F(1, 25) = 13.09, p < 0.01$ (Table 1). A previous study has demonstrated that similar doses of atropine block carbachol and ACh-induced polydipsia (20).

Previous studies have demonstrated that the prostration response induced by MCC is abolished by pretreatment with nicotinic receptor antagonists such as DHBE (42). Thus, it appears that ICV injections of MCC induce behaviors that are regulated by both muscarinic and nicotinic cholinergic receptors. Because chronic MCC administration is associated with the development of tolerance to MCC-induced prostration syndrome (42), we sought to determine whether tolerance develops to the muscarinic receptor mediated polydipsia produced by MCC. Animals were injected with MCC (30 μg) twice daily for 10 days. Each injection of MCC produced a significant drinking response, although this chronic treatment paradigm did not cause any changes in the overnight water consumption (data not shown). There was no evidence for acute tolerance to MCC-induced polydipsia because there was no significant difference between the drinking induced by the first and second MCC injection of each day (Fig. 2). MCC-induced drinking was not significantly altered on any day of the 10-day chronic treatment regimen, indicating a lack of tolerance development (Fig. 2).

DISCUSSION

The results of this study indicate that methylcarbamylcholine (MCC) produces a physiological response that is mediated through muscarinic cholinergic receptors. This is a significant finding because several studies have previously concluded that MCC is a selective nicotinic cholinergic agonist. We found no evidence suggesting that MCC-induced increases in drinking are mediated through nicotinic receptor stimulation. Pretreatment with nicotinic receptor antagonists such as DHBE, hexamethonium, and mecamylamine did not alter the drinking response to MCC challenge. The doses of these drugs that were used in the present study are comparable to doses that have been reported to block multiple behavioral and physiological effects of nicotinic agonists (1,3,10,13,42). The present data are consistent with the results of Dulaney et al. (14) who reported that water consumption induced by the administration of DFP can be blocked by atropine but not mecamylamine. One study has suggested that water consumption in primates is regulated by nicotinic cholinergic receptors (28). These authors reported that nicotine induced a significant drinking response when administered around the mammillary bodies in the hypothalamus. However, results of the present study and many others (11,15,16,20,33,37), indicate that drinking behavior in rodents is mediated through enhancement of muscarinic rather than nicotinic neurotransmission.

A variety of biochemical, physiological and behavioral data have suggested that MCC is a specific nicotinic cholinergic receptor ligand. However, the muscarinic actions of this drug reported in the present paper are supported by several studies that have examined the pharmacology of MCC binding to brain receptors. The K_i values for displacement of [^{3}H]MCC binding by muscarinic receptor antagonists (including oxotremorine, atropine, and QNB), are about 40–100 μM (2,9). Banerjee and Abood (6) reported that 100 μM MCC inhibits [^{3}H]QNB binding to rodent brain membranes by about 50%. Functional studies also suggest that some of the effects of MCC may be mediated through muscarinic receptors. For example, Boksa et al. (8) demonstrated that MCC-induced contraction of isolated rat ileum is completely inhibited by atropine but not altered by nicotinic receptor antagonists. Thus, it is likely that the concentrations of MCC that were used in the present study activate both muscarinic and nicotinic cholinergic receptors. Identical doses of MCC have been previously shown to induce a characteristic prostration syndrome (that is blocked by nicotinic but not muscarinic receptor antagonists) and also increase the number of brain nicotinic receptors following chronic administration (42). The effects of chronic MCC administration on brain muscarinic receptors remain to be investigated.

In the present experiments, the inverse relationship between the dose of MCC administered and the latency to initiate drinking behavior is explained by the occurrence of MCC-induced prostration responses. Previous experiments demonstrated that MCC at the lowest dose tested (3 μg) causes a short-lived prostration response in about 25% of experimental animals. Thus, majority of the animals that receive this dose of MCC are capable of initiating an immediate drinking response. At higher doses of MCC (30 μg and 60 μg) animals show a prostration response that can include up to 10 min of immobilization (42). Even in the presence of robust prostration responses, the latency for initiation of water drinking is still in the range of that induced by ICV administration of carbachol (16). The effects of both acute and chronic MCC administration had only transient effects on water consumption. Enhanced water consumption was evident for the first hour following MCC administration. The amount of water consumed overnight did not differ between MCC and control-treated animals in both the acute and chronic experiments. Similar findings have been reported following central injections of carbachol and DFP (4,14,16).

It is interesting to note that in the current study there was no evidence for the development of tolerance to MCC-induced changes in drinking. Yang and Buccafusco (42) used a chronic treatment paradigm identical to that described in the present paper and demonstrated two types of tolerance to MCC-induced prostration syndrome. The prostration response following the second daily injection of MCC was always lower in comparison with the first injection in each day, indicating the presence of acute tolerance or tachyphylaxis. Acute tolerance to MCC-induced prostration may be due to agonist-induced desensitization of neuronal nicotinic receptors. Following a chronic treatment regimen, complete tolerance to MCC-induced prostration syndrome was achieved by the seventh day of treatment and was correlated with an increase in the number of brain nicotinic receptors (42). However, no tolerance developed to MCC-induced water consumption (presumably a muscarinic effect) in the present study. Previous studies have demonstrated that chronic administration of other muscarinic receptor agonists results in behavioral tolerance to the effects of acute challenge doses of these agents (21,22,24,32). It is possible that the treatment paradigm used in the present study was not sufficient to induce changes in the number and/or functional status of muscarinic receptors that regulate drinking behavior.

In summary we have demonstrated that central injections of MCC induce a significant increase in water consumption that is dose related and transient. This effect of MCC appears to be mediated by muscarinic rather than nicotinic neurotransmission. Under the proper assay conditions, [^{3}H]-MCC can be used to identify saturable and specific high-affinity binding to nicotinic cholinergic receptors. However, when using this compound for behavioral and biochemical studies, it should be considered that MCC may produce muscarinic as well as nicotinic receptor-mediated events.

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