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Cholinergic Stimulation of Drinking From the Lateral Hypothalamus: Indications for M_2 Muscarinic Receptor Mediation

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HAGAN, J. J., J. A. D. M. TONNAER AND C. L. E. BROEKKAMP. *Cholinergic stimulation of drinking from the lateral hypothalamus: Indications for M_2 muscarinic receptor mediation.* PHARMACOL BIOCHEM BEHAV **26**(4) 771–779, 1987.—Available evidence suggests that muscarinic receptor binding sites may exist in at least two heterogenous subclasses (M_1 and M_2), distinguished by their affinity for the antagonist pirenzepine. In order to evaluate the role of these receptors in comsummatory behaviour a series of conventional and putatively receptor selective drugs were tested for their effects on water consumption following injection (0.5 μl/30 sec) into the perifornical hypothalamic area of non-deprived rats. Of the conventional agonists tested, carbachol and oxotremorine were approximately equipotent and arecoline was about 16× weaker. Of the putative M_1 agonists tested, pilocarpine was about 50× weaker than carbachol and the remainder (MCNA343, AHR602, AH6405) were inactive. Inhibition of carbachol (1 μg) induced drinking was subsequently measured. The most potent inhibition was found using scopolamine, a non selective antagonist. 4-DAMP was approximately 7× weaker than scopolamine, but was more potent than the putative M_1 antagonists pirenzepine, telenzepine or dicyclomine. In a separate series of experiments the affinity of these drugs for [^{3}H]pirenzepine forebrain receptors (M_1) and [^{3}H]QNB brainstem receptors (M_2) was determined to confirm their receptor binding selectivity. No systematic relationship was found between agonist potency and M_1 or M_2 affinities. M_2 receptor involvement was indicated by the antagonist data which show a close relationship between rank potency order and M_2 receptor affinity. An important role for M_1 receptors is excluded by the absence of a clear relationship between potency order and M_1 affinity. The data therefore suggest an important role for M_2 receptors in mediating drinking stimulated by muscarinic receptor activation.

Lateral hypothalamus Drinking M_1/M_2 muscarinic receptors Cholinergic agonists/antagonists

IN his classic studies Grossman [19,20] showed that non-deprived rats would drink vigorously in response to crystalline carbachol placed in the lateral hypothalamus. Nonspecific vascular and osmotic actions did not account for this effect [19,20] and the failure of carbachol to stimulate eating [19, 20, 32, 40] suggested a degree of behavioural specificity and argued against mediation through a more generalised effect on arousal. Cholinergic stimulation of drinking was quickly confirmed [32,40] and additional studies showed that mediation was via muscarinic rather than nicotinic receptors [40]. Noradrenergic [19, 20, 32] serotonergic [40] and dopaminergic [41] neurons appeared not to be involved.

A re-evaluation of the cholinergic hypothesis of drinking was prompted by studies showing that muscarinic receptor binding sites may exist as at least two heterogenous subclasses (M_1 and M_2) (for reviews see [5, 14, 45]). The atypical antagonist pirenzepine has high affinity for M_1 receptors [7, 22, 23] which are mainly found in the cortex, neostriatum, nucleus accumbens and hippocampus of rat [10, 39, 44] and human brains [11,26]. M_2 receptors have a low affinity for pirenzepine receptor sites and are also found in these forebrain areas, but dominate the thalamus and brain stem [10, 11, 26, 39, 44]. Agonist [4] and antagonist [22,23] binding studies, electrophysiological [12, 13, 27,33], lesion [30] and biochemical [31,35] data support the heterogeneity concept, but, with the exception of preliminary data [8], behavioural evidence is lacking.

To test the functional significance of muscarinic receptor subtypes in the expression of drug induced drinking, carbachol, oxotremorine and arecoline were compared with the putative M_1 agonists pilocarpine [9], MCNA343 [3, 23, 33, 38], AHR602 [17] and AH6405 [29] for which receptor selectivity has been claimed on the basis of *in vivo* and *in vitro* data. Potency for inhibiting carbachol (1 μg) induced drinking was subsequently determined for a series of antagonists. Scopolamine, a non-selective antagonist was compared with the ileal selective drug 4-DAMP [1,2] and with pirenzepine [22, 23,42], telenzepine [15,42] and dicyclomine [24,42] all chosen on the basis of data indicating high M_1 selectivity. Measurements of food consumption were included in all experiments in order to exclude the possibility that drug induced side effects interfere with the expression of

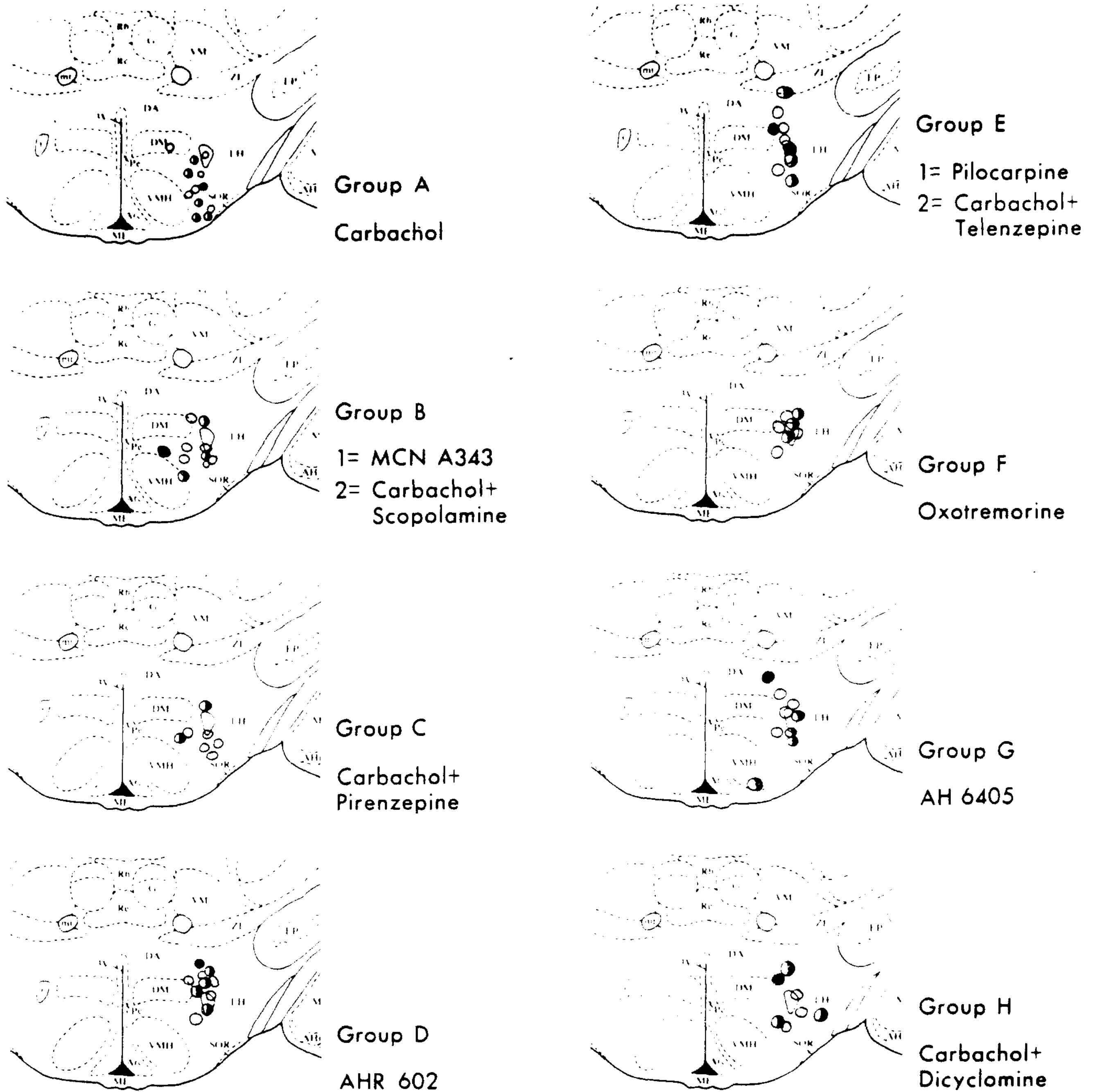

FIG. 1. Cannula tracks were reconstructed from serial brain sections. Deepest penetration points were determined and are depicted for each experimental group. Water consumption in response to carbachol (1 μg/rat) is shown: ○=5–10 ml/90 min; ◑=10–15 ml/90 min; ●=>15 ml/90 min. Sections are taken from Paxinos and Watson [34] at 6.2 mm anterior to EBO. ZI=zona incerta; LH=lateral hypothalamus; F=fornix; VMH=ventromedial hypothalamic nuc.; DM=dorsomedial hypothalamic nuc.; DA=dorsal hypothalamus.

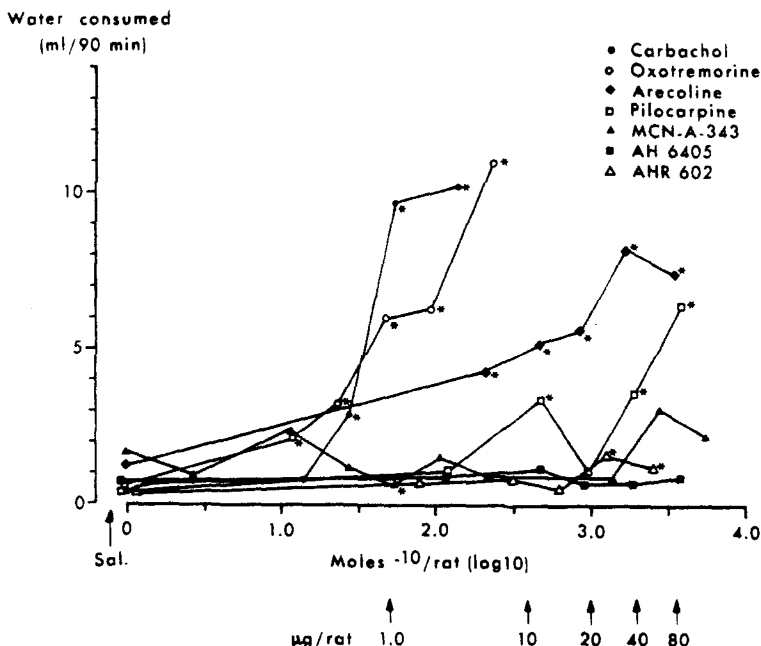

FIG. 2. Agonist doses (expressed as log M^{-10}) are plotted against mean water intake during the 90 min following injections in the perifornical region of the lateral hypothalamus. Approximate scale for doses, expressed as µg/rat, is also shown. For carbachol group n=15, otherwise n=9–10 (see Table 1), $*p<0.05$. SAL=saline.

agonist or antagonist activity. In a separate series of experiments agonist and antagonist affinities for M_1 and M_2 receptor sites were determined by displacement of [^{3}H]pirenzepine from rat forebrain homogenates (M_1) and of [^{3}H]QNB from rat brainstem homogenates (M_2). These were then compared with rank orders of potency derived from the drinking experiments.

Drinking has been reported following stimulation of a number of sites in the rat CNS including limbic and other extra-hypothalamic areas [16, 21, 25, 37]. Although multiple sites of action may exist, the perifornical area is nevertheless a region which is highly sensitive [19, 20, 32, 40] and was therefore selected as suitable for pharmacological comparisons.

METHOD

Subjects

Adult male Cpb:WU rats (TNO; Zeist) were housed singly in a temperature controlled environment (22±1°C) with 12 hour light/dark cycle (lights on 6 a.m. to 6 p.m.). Ad lib food and water were available throughout the experiments.

Surgery

Rats (200–220 g) were anaesthetised with Nembutal® (1 ml/kg; 60 mg/ml) and placed in a stereotaxic frame with the incisor bar positioned 2.4 mm below the interaural line with lambda and bregma in the same horizontal plane [34]. A stainless steel cannula (10.5 mm long × 0.65 mm external diameter) was implanted in the left lateral hypothalamus at the following co-ordinates: AP + 5.7 mm anterior to earbar zero (EB0); LAT + 1.1 mm from midline; H + 1.6 mm above EB0. Four screws were placed in the surrounding skull, the cannula was embedded in carboxylate cement and the wound closed and dressed with antibiotic (Sterilon®). A stainless steel stylet, cut the same length as the cannula, was inserted to keep the cannula patent until use.

Drugs and Injection Procedure

After removing the stylet an intrahypothalamic injection was made using a stainless steel syringe which extended up to 0.1 mm past the tip of the guide cannula. The injection volume was 0.5 µl, delivered over 30 sec by a Hamilton 10 µl syringe connected via plastic tubing (Technicon®) to a CMA®/100 microinfusion pump. Exceptions to these parameters are noted in the text. The syringe was left in place for approximately 15 sec after each injection and the stylet was then replaced.

Drugs were freshly prepared each day in artificial cerebrospinal fluid (CSF) and the pH was adjusted to approximately 7.0 except where noted. The following agonists were tested: carbachol (0.025, 0.05, 0.25, 0.5, 1.0, 2.5 µg/rat); MCNA343 (0.087, 0.38, 0.87, 1.74, 3.48, 6.96, 43.55, 86.5, 173.1 µg/rat); oxotremorine (0.25, 0.5, 1.0, 2.0, 5.0 µg/rat); pilocarpine (2.5, 10, 20, 40, 80 µg/rat); AHR602 (2.5, 10, 20, 40, 80 µg/rat); AH6405 (2.5, 10, 20, 40, 80 µg/rat); arecoline (5, 10, 20, 40, 80 µg/rat). Doses of 80 µg/rat and more were delivered in 1 µl over a one min period. The highest dose of MCNA343 (173.1 µg/rat) was delivered as 2 µl/2 min.

The following antagonists were tested: scopolamine hydrochloride (0.08, 0.83, 3.33 µg/rat); 4-DAMP (0.1, 1, 10, 20, 32 µg/rat; pirenzepine (0.12, 0.61, 1.22, 2.43, 9.7, 38.8 µg/rat); telenzepine (8.88, 35.5, 71.0 µg/rat); dicyclomine (1.9, 7.53, 15.2, 45.7 µg/rat). For antagonist experiments the drug was mixed with a carbachol (1 µg/rat) solution to yield the correct

TABLE 1

THE EFFECTS OF CHOLINERGIC AGONISTS ON FOOD CONSUMPTION (CM OF SPAGHETTI) DURING THE 90 MINUTES FOLLOWING INJECTION INTO THE PERIFORNICAL REGION OF THE LATERAL HYPOTHALAMUS IN SATIATED RATS

Dose (μg/rat)	Carbachol n=15	Oxotremorine n=9	Arecoline n=9	Pilocarpine n=8	AHR602 n=10	AH6405 n=9	MCNA343 n=9
CSF	17.1	41.0	38.0	8.8	19.3	5.1	61.0
0.025	63.7*	—	—	—	—	—	—
0.05	25.2	—	—	—	—	—	—
0.087	—	—	—	—	—	—	76.8
0.25	29.7	28.9	—	—	—	—	—
0.38	—	—	—	—	—	—	75.8
0.50	32.5	32.3	—	—	—	—	—
0.87	—	—	—	—	—	—	49.6
1.00	53.1	45.1	—	—	—	—	—
1.74	—	—	—	—	—	—	78.9
2.0	—	67.0	—	—	—	—	—
2.5	67.9*	—	—	21.3	14.9	17.1*	—
3.48	—	—	—	—	—	—	80.2
5.0	—	49.8	48.0	—	—	—	—
6.96	—	—	—	—	—	—	65.9
10.0	—	—	65.7	28.7*	28.8	15.3*	—
20.0	—	—	39.6	15.3	24.6	30.2*	—
40.0	—	—	54.9	24.7*†	37.1	31.6*	—
43.5	—	—	—	—	—	—	76.4†
80.0	—	—	62.2	51.1*†	32.3	50.9†	—
86.5	—	—	—	—	—	—	131*†
173.0	—	—	—	—	—	—	82.2‡

*$p<0.05$.
†Injection 1 μl/min.
‡Injection 2 μl/min.
—Not tested.
Data are geometric means.

dose in a final volume of 0.5 μl. The injection volume for the highest dose of dicyclomine and telenzepine was 1.0 μl. Dicyclomine solutions were used at pH 5.0 as precipitates formed at pH 7.0 when this drug was used in the highest concentrations.

Procedure

During the two week post-operative recovery period normal lab diet was supplemented once a day with two sticks of dry spaghetti in order to habituate the rats to this novel food. They were then adapted to the test cages for 24 hours. These were identical to the home cages (clear perspex: length 37 cm, width 21 cm, height 15 cm) with sawdust litter. Water was available through a calibrated glass pipette (25 ml: 0.1 ml divisions) and dry spaghetti through a glass tube dispenser. Ordinary lab food was not provided in the test cages. Most rats drank normal amounts of water (20–26 ml) and ate spaghetti during the 24 hour adaptation period. The procedure was repeated for those which did not.

Drug Testing

When habituation of all rats was completed they were tested for their sensitivity to carbachol before allocation to experimental groups. Each rat was placed in a test cage for 90 min with water and spaghetti available. During this pretest period the animals were initially active, later becoming quiescent. After 90 min the rat was removed, injected with 1 μg of carbachol in 0.5 μl artificial CSF (pH 7.0) according to the procedure outlined above and returned to the test cage. Water consumption was measured after 90 min and those rats which drank more than 4 ml (approximately 85%) were included in the experiment. On the basis of their responses to carbachol, rats were assigned to matched experimental groups.

Design and Statistics

Each rat was used in a maximum of two experiments and within a particular experiment received each dose of the test drug plus placebo. Treatment orders were allocated using a latin square and injections were separated by at least 48 hours. Test sessions consisted of 90 min habituation followed by an injection. Water and spaghetti intake were then measured after a further 90 min. Total water and spaghetti consumption data were transformed (log 10) and analysed using repeated measures analysis of variance. When significant treatment effects were detected 95% confidence limits were calculated to determine which treatment conditions differed significantly from placebo.

ED_{50} values were calculated by linear regression as the dose of agonist required to stimulate 5 ml of water consumption. Antagonist ED_{50} values were calculated as the dose of antagonist required to inhibit carbachol (1 μg) stimulated drinking by 50%.

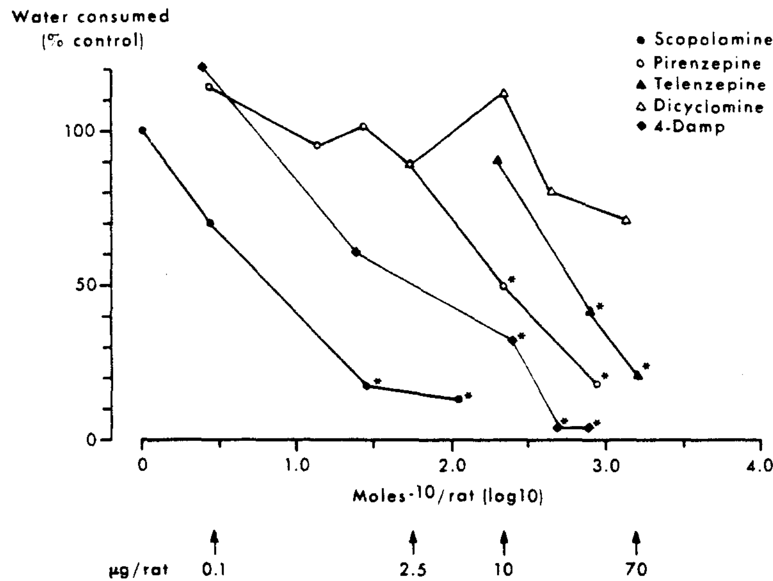

FIG. 3. Carbachol (1 μg/rat) was combined with various doses of antagonists and the % inhibition of drinking determined. One hundred percent represents approximately 8–11 ml of water consumed in the 90 minutes after injections. The approximate scale for doses, expressed as μg/rat, is also shown. In each group n=6–10. *$p<0.05$.

Histology

At the end of the experiments rats were deeply anaesthetised with nembutal and perfused transcardially with physiological saline and 4% paraformaldehyde. Brains were then removed and stored in 4% paraformaldehyde until sectioned. Sections (32 μM) were saved at intervals of approximately 80 μM and stained with cresyl violet. Cannula tracks were then reconstructed and the deepest point of penetration was estimated. These are shown in Fig. 1. Most cannula tips were located between approximately 5.7 and 6.2 mm anterior to Earbar 0 [34] within the lateral hypothalamus, adjacent the perifornical region.

Receptor Binding

In a separate series of experiments affinity for CNS M_1 and M_2 receptors was determined (see Table 3). The methods have been fully described elsewhere [42]. Briefly, affinity for the M_1 site was measured by displacement of [^{3}H]pirenzepine from receptors in tissue homogenates prepared from the rat forebrain [10, 11, 44, 45]. M_2 receptor affinity was measured by displacement of the non-selective ligand [^{3}H]QNB from homogenates of rat brainstem, which are known to contain few high affinity [^{3}H]pirenzepine (M_1) sites [10, 11, 44, 45]. Membranes (approximately 0.2 mg of protein/ml) from rat forebrain or rat brainstem were incubated for 60 minutes at 25°C in 50 mM phosphate buffer (pH 7.4) with [^{3}H]pirenzepine (0.1 nM) or [^{3}H]QNB (0.6 nM) respectively, in the presence of at least 6 concentrations of competing drugs (10^{-11}–10^{-4} M). Nonspecific binding of [^{3}H]pirenzepine and [^{3}H]QNB was determined in the presence of 1 μM atropine. Following arc-sinus transformation of the displacement data, IC_{50} values were estimated by linear regression and K_i values calculated. Binding assay data are summarised in Table 3.

RESULTS

Informal observations suggest that the latency to the onset of drinking was in the range of 2–6 min. Rats would approach the drinking spout and drink vigorously for several seconds, repeating such bouts at various intervals up to approximately 60 min after injection. Carbachol, in its highest dose (2.5 μg/rat) induced brief seizures in some rats, but otherwise no abnormal behaviour was observed in either the agonist or antagonist studies.

Dose response curves for each of the agonists are shown in Fig. 2. Water intake was significantly increased by carbachol in doses of 0.5 μg/rat and greater ($p<0.05$).

Oxotremorine also increased water intake even at the lowest dose tested (0.25 μg/rat; $p<0.05$) suggesting a lower threshold than in the case of carbachol. However, when estimated as the dose required to elicit 10 ml of water consumption oxotremorine was approximately 3× weaker than carbachol. Arecoline stimulated drinking at all doses tested ($p<0.05$) but the dose-response curve was flatter than in the case of carbachol. Pilocarpine stimulated drinking after 10 μg/rat ($p<0.05$) but reliable dose dependent increases were not observed until doses of 40 or 80 μg/rat were administered. MCNA343 failed to elicit drinking at any dose tested although a very small but significant decrease was observed after 1.74 μg/rat ($p<0.05$). AH6405 was also inactive. Small but significant increases in drinking were found after the highest doses of AHR602 (40, 80 μg/rat), but these were not dose related.

For the four agonists which reliably induced drinking (carbachol, oxotremorine, arecoline and pilocarpine) the dose required to stimulate consumption of 5 ml was calculated and their potency expressed relative to carabachol (see Table 3).

The effects of agonists on spaghetti consumption are

TABLE 2

THE EFFECTS OF CHOLINERGIC ANTAGONISTS ON FOOD CONSUMPTION WHEN COMBINED WITH CARBACHOL (1 μg/RAT) AND INJECTED INTO THE PERIFORNICAL REGION OF THE LATERAL HYPOTHALAMUS OF SATIATED RATS

Antagonist Dose (μg/rat)	Scopolamine n=9	4-DAMP n=8	Pirenzepine n=10	Telenzepine n=9	Dicyclomine n=6
CSF	66.3	35.8	72.6	19.2	16.4
0.08	77.1	—	—	—	—
0.10	—	25.5	—	—	—
0.12	—	—	52.5	—	—
0.61	—	—	75.7	—	—
0.83	71.1	—	—	—	—
1.0	—	34.0	—	—	—
1.22	—	—	48.2	—	—
1.90	—	—	—	—	47.5*
2.43	—	—	71.8	—	—
3.33	51.5	—	—	—	—
7.53	—	—	—	—	44.1*
8.88	—	—	—	12.0	—
9.7	—	—	68.3	—	—
10.0	—	16.7	—	—	—
15.2	—	—	—	—	22.4
20.0	—	5.68*	—	—	—
32.0	—	7.96*	—	—	—
35.5	—	—	—	5.0	—
38.8	—	—	73.7	—	—
45.7	—	—	—	—	43.1*†
71.0	—	—	—	8.9	—

*$p<0.05$.
†Injection volume 1 μl/min.
—Not tested.
Data are geometric means.

summarised in Table 1. Carbachol did not induce dose related increases although there were non systematic differences and consumption was significantly greater after the lowest (0.025 μg/rat) and the highest (2.5 μg/rat) doses ($p<0.05$). Neither oxotremorine, arecoline nor AHR602 altered spaghetti consumption and MCNA343 was inactive except for a moderate increase after a single dose (86, 5 μg/rat, $p<0.05$). AH6405 increased consumption at all doses tested (all p values <0.05) with some suggestion of dose dependency. Pilocarpine increased spaghetti consumption at doses (10, 40, 80 μg/rat) which also increased water intake (all p values <0.05). However, in both the pilocarpine and AH6405 experiments control levels of spaghetti consumption were amongst the lowest observed throughout the series of experiments. The finding of significant stimulation of feeding with these drugs is therefore contaminated by unusually low control levels of food intake.

Antagonist effects on carbachol induced drinking are depicted in Fig. 3. Scopolamine was the most potent antagonist, significantly inhibiting water intake at 0.83 and 3.33 μg/rat ($p<0.05$), but not affecting spaghetti consumption.

4-DAMP (10, 20, 32 μg/rat) also inhibited carbachol induced drinking ($p<0.05$) but the two highest doses were associated with reduced spaghetti intake ($p<0.05$). Significant dose-dependent antagonism of carbachol induced drinking was found with both pirenzepine (9.7, 38.8 μg/rat) and telenzepine (35.5, 71.0 μg/rat) (all p values <0.05) without significant effects on spaghetti intake (Table 2). In the case of dicyclomine 30% inhibition of drinking approached significance ($p<0.1$) after the highest dose (45.7 μg/rat), but significant increases in food intake were also seen after this and two lower doses (1.9, 7.53 μg/rat) (all p values <0.05). The dose required to induce 50% inhibition of drinking was calculated for all four antagonists (see Table 3).

DISCUSSION

The main finding of the agonist studies was that putative M_1 selective agonists were either very weak or inactive in eliciting drinking. In contrast, carbachol stimulated dose-dependent drinking, confirming previous reports [19, 20, 32, 40], whilst not affecting food consumption [19,20]. Oxotremorine was approximately equipotent to carbachol when compared at the dose required to elicit consumption of 5 ml of water, but had a slightly shallower dose response curve. Arecoline also stimulated drinking but, as in the case of oxotremorine, the dose response curve was flatter than for carbachol. Dose-dependent stimulation of drinking was not found with the putative M_1 agonist pilocarpine until 40 μg or more were injected. According to the ED_{50} values pilocarpine was approximately 50 fold weaker than either oxotremorine or carbachol. MCNA343 and AH6405, two more putative M_1 agonists, were completely inactive and the weak effect of AHR602 at high doses was not dose dependent.

TABLE 3

AFFINITIES OF SELECTED AGONISTS AND ANTAGONISTS FOR M_1 RECEPTORS ([^{3}H]PIRENZEPINE) IN THE RAT FOREBRAIN AND M_2 RECEPTORS ([^{3}H]QNB) IN RAT BRAIN STEM

Drug	[^{3}H]Pirenzepine pK_i	[^{3}H]QNB pK_i	ED_{50}† (log M^{-10})	Potency (%)
		Agonists		
Carbachol	4.4	6.1	1.52 ± 0.05	100
Oxotremorine	6.7	7.4	1.49 ± 0.10	107
Arecoline	—	—	2.75 ± 0.12	5.8
Pilocarpine	6.1	6.1	3.22 ± 0.14	1.9
MCNA343	5.8	5.3	—	—
AHR 602	5.9	5.3	—	—
AH 6405	5.0	5.1	—	—
		Antagonists		
Scopolamine	9.4	9.3	0.93 ± 0.09	100
4-DAMP	9.0	8.2	1.82 ± 0.08	12.8
Pirenzepine	8.5	6.6	2.28 ± 0.11	4.46
Telenzepine	9.5	7.9	2.31 ± 0.10	4.16
Dicyclomine	11.9*	7.6	3.29 ± 0.22	0.44

*Dicyclomine interacted atypically with [^{3}H]pirenzepine receptors (Hill coefficient=0.35).

†ED_{50} values were calculated by linear regression as the dose of agonist required to stimulate 5 ml water consumption or the dose of antagonist required to inhibit carbachol (1 μg/rat) stimulated consumption by 50%. Data shown are group means (±SEM). Potency calculated with respect to carbachol and scopolamine.

—Not tested.

Corresponding pharmacological potencies for eliciting drinking (agonists) and for inhibiting carbachol stimulated drinking (antagonists) are also shown.

A number of factors may be excluded in accounting for these potency differences. First, the pre-selection of rats, on the basis of their response to carbachol, excludes the possibility that inaccurate cannula placements account for the lack of effect with MCNA343, AHR602 and AH6405 or for the systematically decreasing potencies of carbachol, oxotremorine, arecoline and pilocarpine. Histological assessments also confirmed a uniform distribution of cannula tips in the vicinity of the perifornical area in all groups. Second, it is unlikely that drinking was stimulated by these drugs but that its expression was blocked by interfering behavioural effects. Adverse behavioural reactions were not seen after the three inactive agonists and the lack of effects on spaghetti consumption following MCNA343 and AH6405 argues against the hypothesis that gross behavioural impairments or sedation masked the effective expression of drinking.

Pharmacological studies in peripheral nervous system preparations [18] show that carbachol is moderately selective for M_2 receptors. This was paralleled in our experiments by a selective M_2 binding affinity in brain stem homogenates, which was also seen in the case of oxotremorine. Although this evidence for selectivity, coupled with the potency of these agonists, may suggest M_2 rather than M_1 mediation of the drinking response, two important factors undermine this conclusion. First, the proposed M_1 selectivity of pilocarpine [18] and AH6405 was not confirmed in the binding studies and in the case of MCNA343 and AHR602 selectivity was only in the order of 3 to 4 fold. The lack of agonist selectivity in binding assays and failure to observe systematic relationships between potency and affinity does not, however, totally undermine the proposal of M_2 mediation as the agonist may still be selective *in vivo*. Indeed, agonist affinity is generally a poor predictor of potency. For example, in the case of oxotremorine, the structural requirements for affinity and potency are known to differ [36].

A second and more serious objection is that in a range of pharmacological and biochemical tests the putative M_1 agonists exhibit low efficacy and behave as partial agonists. Differences in efficacy, due to variations in intrinsic activity or the efficiency of receptor coupling, may give a false impression of receptor specificity when agonists are compared, especially in tissues with low receptor reserve [44]. Some of these interpretive difficulties may be avoided when antagonist data are used as a basis for receptor classification.

Except at the highest doses of 4-DAMP food intake was not suppressed by antagonists. This rules out an explanation of the inhibition of carbachol induced drinking in terms of gross behavioural disruption or sedation. There is good agreement from binding studies that scopolamine fails to differentiate M_1 and M_2 sites [22, 23, 42] and our data (see Table 3) confirm this conclusion. A moderate selectivity for M_1 receptors was found in the case of 4-DAMP [1,2]. Pirenzepine, telenzepine and dicyclomine were confirmed as selective M_1 antagonists although in the case of dicyclomine the interaction with the [^{3}H]pirenzepine binding site was atypical. In terms of inhibiting carbachol stimulated drinking, scopolamine was the most potent antagonist. This is not

surprising as, although lacking selectivity, scopolamine nevertheless has very high affinity for both receptor subtypes. Data from the antagonist experiments rule out an important M_1 role in the inhibition of drinking. No systematic relationship was apparent between the rank orders of antagonist potency (scopolamine > 4-DAMP > pirenzepine > telenzepine > dicyclomine) and M_1 binding affinities (dicycl > telenz > scop > pirenz > 4-DAMP). Furthermore telenzepine, although 4–10× more potent than pirenzepine at peripheral M_1 receptors [15] was actually slightly weaker than pirenzepine in our test. Finally, if dicyclomine is excluded on the basis of its atypical interaction at M_1 sites, then large potency differences (230 fold) are seen to accompany very modest differences in M_1 affinity (10 fold).

In contrast, the rank potency order (scopolamine > 4-DAMP > pirenzepine > telenzepine > dicyclomine) is closely related to the rank order of M_2 binding affinities (scopolamine > 4-DAMP > telenzepine > dicyclomine > pirenzepine). The only exception to a linear relationship is the position of pirenzepine which appears anomalously potent for a relatively low binding constant. Clearly, M_2 affinity alone does not provide a perfect predictor of potency but in such in vivo experiments, where equilibrium conditions cannot be achieved, other factors may introduce distortions. For example, the low lipophilicity of pirenzepine (J. Kelder, personal communication) may result in a slower diffusion of the drug away from the active receptors, thereby increasing the apparent potency in comparison to the more lipophillic drugs, scopolamine HCl and dicyclomine.

In summary, our data show that potent agonists such as carbachol and oxotremorine elicit vigorous drinking when injected into the lateral hypothalamus but putatively M_1 selective agonists are either much weaker (pilocarpine) or are inactive (MCNA343, AHR602, AH6405). An argument in favour of M_2 mediation is supported by the finding that there is close agreement between the antagonist rank potency order and M_2, but not M_1, receptor affinity. Our procedure may provide a sensitive test for M_2 selective drugs which are active in the brain, however it requires further validation with more selective M_2 antagonists and potent M_1 agonists when these become available.

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