Biochemical Pharmacology, Vol. 31, No. 9, pp. 1693–1698, 1982.
Printed in Great Britain.

0006-2952/82/091693-06 $03.00/0

EFFECT OF DIMETHYLAMINO-2-ETHOXYIMINO-2-ADAMANTANE (CM 54903), A NON-POLAR DIMETHYLAMINOETHANOL ANALOG, ON BRAIN REGIONAL CHOLINERGIC NEUROCHEMICAL PARAMETERS

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(*Received* 28 *July* 1981; *accepted* 7 *December* 1981)

Abstract—CM 54903, a new psychotropic drug with a particular pharmacological profile, produced a widespread but short-lasting decrease in acetylcholine content in rat brain hemispheric regions but not in the midbrain–hindbrain or cerebellum at the dose of 40 mg/kg, i.p. The decrease was most conspicuous in the striatum. Brian regional choline contents were unaltered as were the acetylcholine turnover rates in the striatum and hippocampus. Neither choline acetyltransferase nor acetylcholinesterase activities were altered after the *in vitro* incubation or the *in vivo* administration of high amounts of the drug. CM 54903 was found to be a competitive, reversible inhibitor of the sodium-dependent high affinity uptake of choline by crude hippocampal and striatal synaptosomal preparations showing an IC_{50} of 10 μM *in vitro*. Despite the fact that the drug readily crosses the blood–brain barrier and achieves brain concentrations several-fold greater than its *in vitro* IC_{50}, CM 54903 did not inhibit choline uptake *in vivo* although it was capable of preventing the pentylenetetrazol-stimulated choline uptake by hippocampal synaptosomes. The changes in striatal acetylcholine content induced by the blockade or the stimulation of muscarinic cholinergic receptors or dopaminergic receptors did not interfere with the effect of CM 54903 on striatal acetylcholine content while pentylenetetrazol completely prevented the decrease. The results taken together indicate that the major effect of CM 54903 on the cholinergic neurons is at the presynaptic level to compete with choline at its uptake sites.

CM 54903 is an analog of dimethylaminoethanol with a hydrophobic substitution at the hydroxyl terminal (Fig. 1) and thus is reminiscent of a choline analog. The compound is a new psychotropic drug with a particular pharmacological profile. It antagonizes aggressive activity in mice and is devoid of muscle relaxant activity. CM 54903 is also very active in antagonizing the catalepsy induced by prochlorperazine and in improving memory performance in experimental animals (unpublished data from Clin-Midy, Montpellier, France).

Little is known about its neuropharmacological actions. CM 54903 does not alter brain noradrenaline, serotonin or dopamine levels in rats but does exert an influence on the dopaminergic system as it increases striatal dihydroxyphenylacetic acid, a metabolite of dopamine, although feebly (S. Algeri, personal communication).

It was therefore decided to assess the effect of CM 54903 on the central cholinergic system in view of the possibility that acetylcholine (ACh) may be involved in some aspects of memory [1, 2] and considering that several psychotropic agents affect brain cholinergic parameters [3, 4].

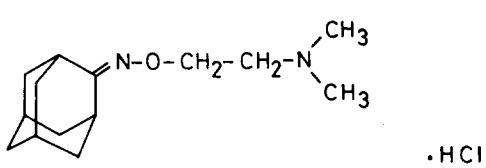

Fig. 1. Structural formula of CM 54903, dimethylamino-2-ethoxyimino-2-adamantane HCl.

MATERIALS AND METHODS

Female CD_1 rats (Charles River, Italy), 180–200 g were used. The animals were housed at constant temperature (23°) and humidity (60%) under fixed 12 hr light and dark cycles for at least four days prior to the experiment. All the experiments were performed during the morning hours to avoid possible circadian variations. The rats were killed by focussed microwave irradiation to the head (1.3 kW, 2.45 GHz for 4 sec). The brain was removed quickly and dissected into different areas (striatum, hippocampus, hemispheric residuum, midbrain–hindbrain and cerebellum. The tissues were homogenized in a solution of 15% 1N formic acid–85% acetone before proceeding to the measurement of ACh and choline by the radiochemical method of Saelens *et al.* [5] with modifications [6].

For the measurement of the acetylcholine turnover rate (TR_{ACh}), a tracer amount of [methyl-^{3}H]choline chloride (80 Ci/mmole, Radiochemical Centre, Amersham, U.K.) was infused into the tail vein of the rat at a constant rate of 0.84 nmole/80 μl/min for 4 min. At the end of this time the rats were killed by focussed microwave irradiation to the head and

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the method of Saelens *et al.* [5] was followed up to the paper electrophoretic step. The [methyl-^{3}H]choline and acetyl[methyl-^{3}H]choline formed were identified by their relative positions to tetraethylammonium on each strip of paper. Their bands were cut out and disintegrated in 1 ml of soluene before adding 0.5 ml of distilled water and 10 ml of toluene base phosphor for scintillation counting. The turnover calculation was determined as described by Racagni *et al.* [7] and done by computer.

Choline *o*-acetyltransferase activity was measured by a modified radiochemical method of McCaman and Hunt [8] and acetylcholinesterase activity was determined by the method of McCaman *et al.* [9]. Sodium-dependent high affinity uptake of choline (SDHACU) was measured by the method of Atweh *et al.* [10] followed by filtration through 0.65 μ Sartorius cellulose nitrate filters.

Brain and plasma levels of CM 54903 were analyzed as follows: brains were homogenized (6 ml/g) in cold acetone–1N formic acid (85:15 v/v), then centrifuged for 15 min; the supernatant was mechanically shaken twice with *n*-heptane–chloroform (4:1). The organic phase was discarded and the aqueous phase was used for drug extraction as described for plasma. 2 ml of plasma were made alkaline by adding 0.1 N NaOH and phosphate buffer (pH 9.5), to a final volume of 5 ml. Each sample was extracted with 10 ml of benzene by shaking for 30 min on an automatic shaker. After centrifugation, the organic phase was evaporated in benzene containing *n*-eicosane as internal marker, and the solution was analyzed by gas chromatography.

A Carlo Erba Fractovap Mod. 61 gas chromatograph with a hydrogen flame detector was used. The chromatographic column was a glass tube 2 m long and 4 mm i.d. packed with 3% OV17 on 100–120 mesh Chromosorb Q. The column temperature was 185° and carrier gas was nitrogen at a flow-rate of 60 ml/min.

Calibration curves for plasma and brain were linear in the range of 0.25 to 5 μg per sample. The minimum detectable amount was about 0.25 μg per sample.

The following drugs, their solvents, routes of administration and dosage schedules were used: CM 54903·HCl was dissolved in distilled water and administered i.p. at the doses and times described in the tables; apomorphine·HCl, 0.7 mg/kg, s.c., 25 min, dissolved in distilled water; oxotremorine sesquifumarate, 0.5 mg/kg, i.p., 20 min, dissolved in distilled water; atropine sulfate, 5 mg/kg, i.p. 35 min, dissolved in distilled water; haloperidol, 1 mg/kg, i.p., 60 min, dissolved in 5 mM HCl; pentylenetetrazol was dissolved in distilled water and administered at doses and times described in the appropriate tables.

Table 2. Dose dependence of the decrease in striatal acetylcholine by CM 54903

Dose of CM 54903 (mg/kg)	Striatal acetylcholine (nmoles/g)
Control	66.6 ± 2.2 (6)
20	53.1 ± 3.5 (6)†
40	40.9 ± 2.5 (8)*
60‡	29.6 ± 1.0 (8)*
80‡	29.2 ± 1.1 (8)*

The data are means ± S.E. (*n*).

* $P < 0.01$; † $P < 0.05$ vs controls; Dunnett's test.

The animals were killed 30 min after the intraperitoneal administration of CM 54903.

‡ These doses caused clonic convulsions.

RESULTS

The effect of CM 54903 on the ACh levels in various brain regions at 30 min following the dose of 40 mg/kg is shown in Table 1. The ACh content was decreased in the striatum (36%), hippocampus (24%) and hemispheric residuum (38%) and was not affected in the cerebellum and midbrain–hindbrain. In terms of the net decrease in ACh content, in nmoles/g, the striatum is the area most sensitive to the action of the drug. The choline content was not altered in any brain region (data not shown but see Table 4 for striatum and hippocampus).

The chronic administration of CM 54903 (40 mg/kg, i.p., twice daily for 7 days) did not result in tolerance to an acute challenge with the same dose 15 hr after withdrawal of the treatment (data not shown).

The dose–response of CM 54903 on the ACh content of the striatum is given in Table 2. The ACh content was slightly affected by the dose of 20 mg/kg while it was decreased by 38% at 40 mg/kg and by 60% at the convulsant doses of 60 and 80 mg/kg. The subconvulsant dose of 40 mg/kg was used in the subsequent experiments.

Table 1. Effect of CM 54903 on the acetylcholine content in rat brain regions

Region	Acetylcholine (nmoles/g) Controls	Treated
Striatum	68.9 ± 1.8 (8)	44.3 ± 2.2 (8)*
Hippocampus	22.8 ± 0.9 (8)	17.3 ± 1.3 (8)*
Hemispheric residuum	24.9 ± 1.8 (8)	15.5 ± 0.9 (8)*
Midbrain–hindbrain	27.1 ± 0.8 (7)	27.0 ± 0.7 (7)
Cerebellum	5.5 ± 0.4 (7)	5.0 ± 0.3 (6)

The data are the means ± S.E. (*n*).

* $P < 0.01$; Student's *t*-test. The animals were killed 30 min after the intraperitoneal administration of CM 54903, 40 mg/kg.

Table 3. Time-course of the decrease in striatal acetylcholine content provoked by CM 54903 and its relation to plasma and whole brain levels of CM 54903

Time after CM 54903 (min)	Striatal acetylcholine† (nmoles/g)	Brain CM 54903‡ (nmoles/g)	Plasma CM 54903‡ (nmoles/g)
Control	65.5 ± 1.6 (6)	—	—
5	—	19.8 ± 2.8	13.2 ± 0.6
15	37.7 ± 2.2 (6)*	33.8 ± 1.9	9.5 ± 0.4
30	42.2 ± 3.4 (6)*	44.2 ± 1.7	6.2 ± 0.5
60	49.5 ± 3.1 (6)*	19.0 ± 1.3	2.9 ± 0.2
120	56.4 ± 4.7 (6)	5.5 ± 0.8	0.8 ± 0.1

The data are means ± S.E. (n).
* $P < 0.01$ vs controls; Dunnett's test.
† Dose of CM 54903, 40 mg/kg, i.p.
‡ Dose of CM 54903, 20 mg/kg, i.p.

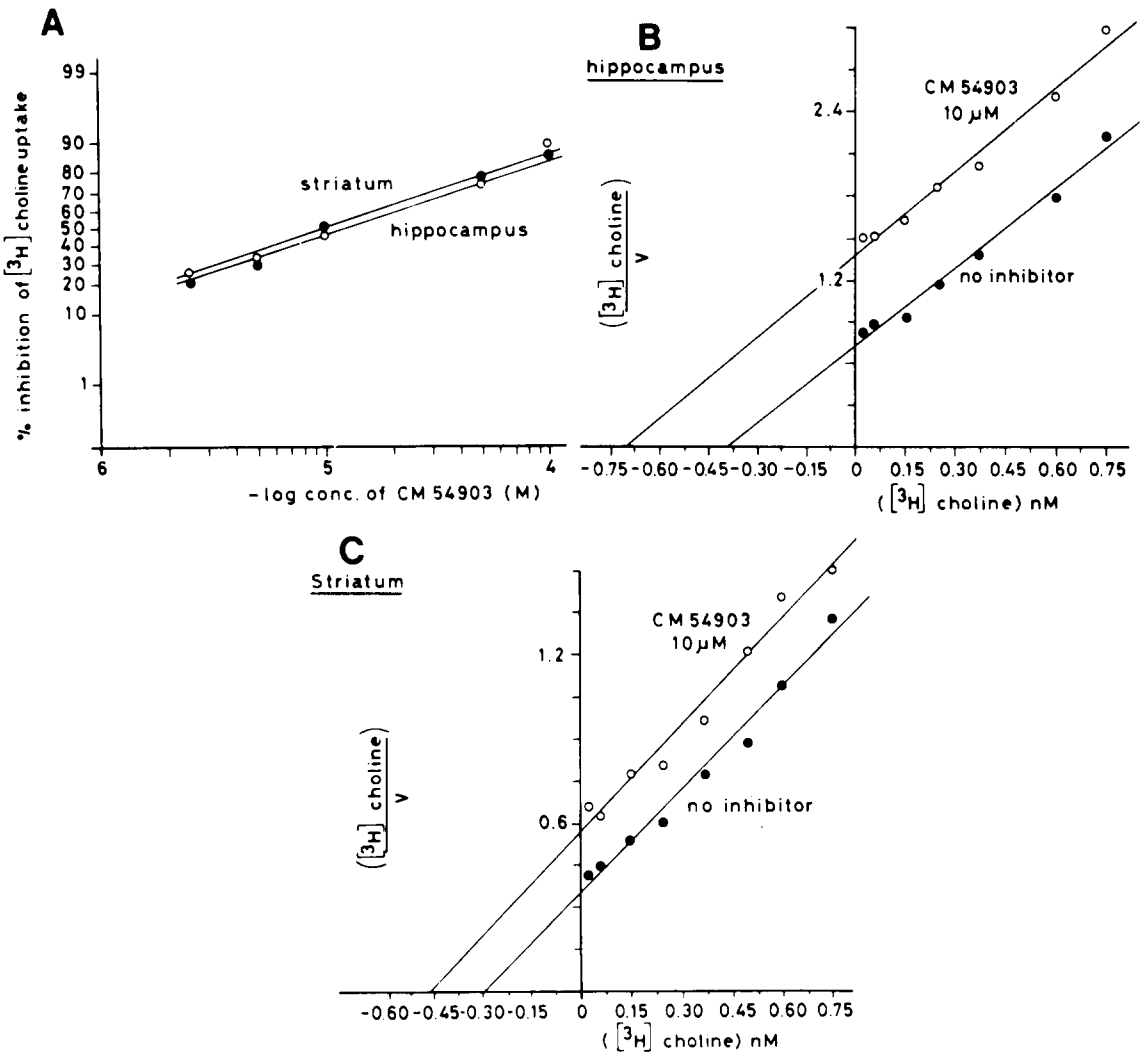

Fig. 2. Inhibition of sodium-dependent high affinity uptake of choline by CM 54903 *in vitro*. (A) Log-probit plots of the inhibitor. Crude synaptosomal fractions from hippocampus and striatum were incubated for 3 min at 30° in the presence of 0.03 μM [methyl-^{3}H]choline (31 Ci/mmole) and various concentrations of CM 54903. The SDHACU values for the control striatal and hippocampal synaptosomes were 40 and 150 nmoles choline/hr/g protein, respectively. The concentration of CM 54903 that inhibited uptake by 50% (IC_{50}) was 10 μM for both tissues. (B) and (C) Woolf plots of the inhibition kinetics of CM 54903 with respect to choline uptake. CM 54903 was found to be a competitive inhibitor of choline uptake in both hippocampus and striatum. V is expressed as nmoles choline taken up per hr per g protein. The V_{max}'s of the inhibitor-free striatal and hippocampal synaptosomal preparations were 630 and 460 nmoles/hr/g protein, respectively.

Table 4. Turnover rates of acetylcholine in rat striatum and hippocampus after a single injection of CM 54903

Region	Treatment	Endogenous ACh (pmoles/mg tissue)	Endogenous Choline (pmoles/mg tissue)	Radioactive ACh (dpm/mg tissue)	Radioactive Choline (dpm/mg tissue)	Specific radioactivity ACh (dpm/pmole)	Specific radioactivity Choline (dpm/pmole)	K_b (hr^{-1})	Turnover ACh (pmoles/min/mg tissue)
Striatum	Saline	66.0 ± 1.3	26.0 ± 1.0	179.9 ± 7.7	203.6 ± 7.5	2.7	7.8	10.4 ± 0.6	11.4 ± 0.6
	CM 54903	42.0 ± 2.8*	23.0 ± 1.3	201.4 ± 11.8	244.7 ± 15.6	4.7	10.6	15.4 ± 1.5*	10.7 ± 1.1
Hippocampus	Saline	22.0 ± 0.8	16.0 ± 0.9	70.9 ± 3.9	190.2 ± 13.2	3.2	11.8	8.9 ± 0.8	3.2 ± 0.3
	CM 54903	17.0 ± 1.3*	16.0 ± 0.8	81.1 ± 4.6	183.5 ± 4.7	4.7	11.4	13.9 ± 1.0*	3.9 ± 0.2

The data are the means ± S.E.M. for 5 animals.
* $P < 0.01$ vs saline group.
CM 54903 was administered i.p. at the dose of 40 mg/kg. The animals were killed by focussed microwave irradiation to the head 30 min after CM 54903 and 4 min after the i.v. infusion of [methyl-^{3}H]choline. This tracer dose did not affect either the ACh content or the choline content of the two brain regions.

A time-course of the effect of CM 54903 showed that the maximal decrease in striatal ACh was achieved within 15 min of its administration (40 mg/kg i.p.) and the effect was maintained for about 30 min before declining and finally terminating by 120 min (Table 3). The brain concentrations of CM 54903 after the i.p. administration of 20 mg/kg, followed a similar time-course (Table 3), with the peak level of 44 nmoles/g being reached at 30 min followed by a rapid fall. The half-lives of CM 54903 in brain and plasma were 32 and 29 min, respectively.

The data reported in Table 4 show that CM 54903 failed to change the ACh turnover rates in striatum and hippocampus.

Striatal choline *o*-acetyltransferase and acetylcholinesterase activities were affected neither by the *in vitro* incubation of the 500 *g* supernatant fraction with CM 54903 up to a final concentration of 10^{-4} M nor after the i.p. administration of the drug at 40 mg/kg, 30 min (data not shown). The enzymic activities were 0.11 mmole ACh synthesized/hr/g protein and 18.1 mmoles ACh hydrolyzed/hr/g protein, respectively, in controls. On the other hand, the drug proved to be an inhibitor of the sodium-dependent high affinity uptake of choline by striatal and hippocampal synaptosomes after *in vitro* incubation, showing an IC_{50} value of 10 μM for both tissues as determined graphically from a logarithmic/probability plot (Fig. 2(A)). Woolf plots of the inhibition kinetics of CM 54903 indicated that the drug is a competitive inhibitor of SDHACU in both brain regions (Fig. 2 (B) and (C)). The inhibition was readily reversed when the inhibited synaptosomes were centrifuged and resuspended in drug-free Krebs–sucrose solution. Despite the fact that its *in vitro* IC_{50} is several-fold lower than its *in vivo* brain concentration (Table 3), the administration of 40 mg/kg of CM 54903 did not affect the SDHACU of striatal (data not shown) or hippocampal synaptosomal P_2 fractions (Table 5). However, this dose of the drug was able to reverse the *in vivo* increase in hippocampal SDHACU produced by a convulsant dose of pentylenetetrazol, 80 mg/kg, 4 min (Table 5).

Table 6 shows the interaction of various types of treatments, known to affect striatal cholinergic activity, with CM 54903. The depletion of catecholamines and serotonin and the partial depletion of ACh by reserpine did not prevent the further depletion of striatal ACh content by CM 54903. In addition, the increase in striatal ACh provoked by the dopaminergic agonist, apomorphine, did not interfere with the decrease in ACh induced by CM 54903. It is to be noted, on the other hand, that CM 54903's decrease in ACh was not completely additive with a more marked decrease induced by a supramaximal dose of haloperidol, as indicated by the significant interaction of the two drugs.

The blockade or the stimulation of muscarinic receptors with atropine or oxotremorine, respectively, did not prevent the effect of CM 54903. By themselves, oxotremorine increased the ACh content by 27% while atropine had no effect on this level.

Pretreatment with pentylenetetrazol at a convulsant dose of 60 mg/kg i.p., 40 min, slightly, but

Table 5. Inhibition of the pentylenetetrazol-induced increase in hippocampal high affinity choline uptake by CM 54903 *in vivo*

Drug	Na^+-dependent high affinity choline uptake (nmoles/g protein/min)
Control	0.52 ± 0.03 (7)
CM 54903	0.60 ± 0.03 (7)
Pentylenetetrazol	0.74 ± 0.02 (7)*
CM 54903 + pentylenetetrazol	0.62 ± 0.02 (7)

CM 54903, 40 mg/kg, i.p., 30 min, pentylenetetrazol, 80 mg/kg, i.p., 4 min.

The data are means ± S.E. (*n*)—ANOVA (2 × 2) factorial analysis, Tukey's test and Tukey's test for unconfounded means.

* $P < 0.01$ vs control.

$F\ 1,24 = 14.85\ P < 0.01$.

significantly, decreased the content of striatal ACh by itself and completely prevented the effect of CM 54903.

DISCUSSION

CM 54903 decreased the level of ACh in the hemispheric structures, i.e. striatum, hippocampus and hemispheric rest. It is known that some classes of drugs including neuroleptics [11], antimuscarinics [12, 13] as well as choline uptake inhibitors [14, 15] can decrease the ACh content. However, CM 54903's effect on striatal ACh was additive with those of the drugs that directly stimulated or blocked muscarinic receptors *in vivo*.

In addition, the data obtained showed that the lowering of striatal ACh did not appear to occur via an action on the dopaminergic system since neither reserpine nor apomorphine interfered with CM 54903. On the other hand, the decrease in ACh produced by CM 54903 did not completely summate with that induced by haloperidol indicating a partial antagonism. Therefore, a minor feature of CM 54903's action is to block dopamine receptors and this notion is supported by the fact that the drug does produce an increase in the metabolism of dopamine. The major effect of CM 54903 on the cholinergic neuron appeared to occur at the presynaptic level to compete with choline at its uptake sites as shown by the competitive inhibition of hippocampal and striatal SDHACU *in vitro*. Since the turnover rate of ACh was not changed, it may be assumed that ACh release, if affected at all by the drug, was not altered to such a degree as to provoke feedback inhibition of the cholinergic neurons. Therefore, inhibition of SDHACU by CM 54903 may provide the means by which the drug lowers ACh. In line with this notion is the *in vivo* mutual antagonism by CM 54903 of the pentylenetetrazol-induced increase in hippocampal SDHACU and the inhibition by pentylenetetrazol of CM 54903's lowering effect on striatal ACh.

It is not clear why CM 54903 failed to alter SDHACU *in vivo*, particularly when considering that the brain concentration of the drug can exceed

Table 6. Effect of pretreatment with various drugs on the CM 54903-induced decrease in striatal acetylcholine content

Drug in columns C and D	Striatal acetylcholine (nmoles/g) A Vehicle	B CM 54903	C Drug	D Drug + CM 54903	Interaction
Reserpine‡	66.2 ± 0.7 (7)	42.0 ± 1.8 (7)*	46.3 ± 3.3 (7)*	31.5 ± 0.8 (7)*	n.s
Apomorphine	66.6 ± 1.6 (6)	47.3 ± 2.8 (5)*	86.2 ± 6.0 (6)*	70.6 ± 4.2 (5)	n.s.
Haloperidol	62.8 ± 2.9 (6)	34.7 ± 2.6 (6)*	41.8 ± 1.8 (6)*	25.8 ± 1.2 (6)*	$F\ 1,20 = 7.06\ P < 0.05$
Oxotremorine	64.4 ± 2.4 (8)	40.8 ± 3.1 (8)*	81.6 ± 1.2 (8)*	70.3 ± 3.0 (7)	n.s.
Atropine	64.9 ± 1.7 (8)	44.1 ± 2.1 (8)*	60.3 ± 2.9 (8)	41.5 ± 3.1 (8)	n.s.
Pentylenetetrazol§	64.7 ± 1.7 (6)	51.9 ± 1.0 (6)*	57.3 ± 2.8 (6)†	57.3 ± 1.4 (6)†	$F\ 1,20 = 11.5\ P < 0.01$

Reserpine, 5 mg/kg, i.p. 16 hr; apomorphine, 0.7 mg/kg s.c., 25 min; haloperidol, 1 mg, i.p., 60 min; oxotremorine, 0.54 mg/kg, 20 min; atropine sulfate, 5 mg/kg, i.p., 35 min; pentylenetetrazol, 60 mg/kg, i.p., 40 min. The rats were killed 30 min after the administration of CM 54903, 40 mg/kg, i.p. The data are means ± S.E. (*n*). The data were analyzed by ANOVA two-way factorial analysis, Tukey's test and Tukey's test for unconfounded means.

* $P < 0.01$ and † $P < 0.05$ vs the vehicle group.

‡ CM 54903 restored motor activity in the reserpinized rats.

§ CM 54903 did not prevent pentylenetetrazol convulsions.

by several fold the *in vitro* IC_{50} concentration. In this regard, CM 54903 behaves similarly to hemicholinium-3 in lowering brain ACh and in inhibiting SDHACU *in vitro* but not *in vivo* [14]. The short half-life of CM 54903 in brain and its easy reversibility of action may be major contributing factors. Thus the drug might be potentially useful as a systematically administered depletor of brain ACh.

More extensive neuropharmacological studies appear to be warranted to elucidate how the cholinergic actions of the drug are related to its pharmacological properties, including its influence on memory performance.

Acknowledgements—We thank Ms. R. Fusi for carrying out the choline uptake studies and Clin-Midy, Montpellier, France, for generously supplying CM 54903.

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