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TRICYCLIC ANTIDEPRESSANTS AS ANTIMUSCARINIC DRUGS: *IN VIVO* AND *IN VITRO* STUDIES

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Abstract—The antagonistic activity of nortriptyline, amitriptyline and imipramine to the hypothermic and tremorgenic activity of oxotremorine was determined in mice. The peripheral anticholinergic relative potencies of these drugs were evaluated by following the dose-dependent time profiles of their mydriatic activity. The binding constants of the antidepressant agent toward the central muscarinic receptor from the mouse whole brain homogenate were determined *in vitro*, and could be correlated with the ED_{50} values found for the three drugs' *in vivo* responses. The three antidepressants tested were found to be 100-fold less active than scopolamine. HBr in all four biological preparations selected. Their anticholinesterase activity towards the enzyme in whole mouse brain homogenate was found to be too low to make any possible contribution to their activity *in vivo*. The relationships between structure and function and the possible contribution of their antimuscarinic property to the observed *in vivo* effects are discussed.

Imipramine and the related tricyclic compounds have been found to be the most useful drugs for the treatment of depression in the last two decades [1–3]. Their therapeutic efficacy has been attributed to an ability to block the uptake of noreprinephrine and 5-hydroxytryptamine into the nerve ending, thereby allowing the transmitters to remain at the receptor sites for a longer period [4, 5]. However, it is known that these drugs show anticholinergic activity in the peripheral and central nervous systems [6–8]. The tricyclic antidepressants are able to prevent hypothermia and tremor caused by the muscarinic agonist oxotremorine in mice [9, 10]. Furthermore, several clinical reports indicate that physostigmine, an anticholinesterase capable of crossing the blood brain barrier, can effectively reverse the anticholinergic CNS manifestations of severe poisoning by the tricyclic antidepressants [11, 12]. In this work we compared the anticholinergic potency of some tricyclic antidepressants as determined *in vitro* by the [^{3}H]-*N*-methyl-4-piperidyl benzilate binding assay in mouse brain homogenate, with data obtained from *in vivo* studies in mice, including the effect of direct injection of the drug on mydriasis, and on antagonism of hypothermia and tremor induced by oxotremorine. Throughout this study amitriptyline, nortriptyline and imipramine were the tricyclic antidepressants and scopolamine served as pure muscarinic anticholinergic agent as reference compound.

MATERIALS AND METHODS

Drugs. The following compounds were dissolved in physiological saline (0.9% NaCl w/v) solution: oxotremorine (Aldrich), nortriptyline HCl (Ikapharm, Israel), amitriptyline·HCl (Asia, Israel), imipramine·HCl (Plantex, Israel), (−) scopolamine·HBr (Plantex). All other drugs used were of analytical purity.

Animals. Adult male mice weighing 18–24 g, ICR strain, were used throughout. Animals were allowed free access to food and drinking water until the commencement of an experiment. Housing and laboratory temperatures were maintained at 23 ± 0.5°. Animals were always injected s.c. with 0.1 ml of drug solution.

Measurement of tremor. Tremor was assessed visually 15 min after 0.2 mg/kg (0.1 ml) oxotremorine s.c. injection, with or without prior injection of an antitremorgenic drug. No attempt was made to grade the severity of the tremor; it was noted as being either present or absent. The percentage of the effect of the drug was defined as the percentage of mice in a group of 10 which showed no tremor up to 15 min after oxotremorine injection.

Measurement of hypothermia. Rectal temperature, measured by an electronic thermometer (YSI), was recorded at regular intervals after 0.1 mg/kg (0.1 ml) oxotremorine s.c. injection, with or without prior injection of an antihypothermic drug. The percentage of the effect was defined as $[(\Delta T_1 - \Delta T_2)/\Delta T_1] \times 100$; where ΔT_1 was the maximal change in temperature after oxotremorine injection, and ΔT_2 was the maximal change in temperature after injection of both oxotremorine and the antihypothermic drug.

Measurement of mydriasis. According to Long *et al.* [13].

Acetylcholinesterase activity. By Ellman's method [14].

Brain uptake of [^{3}H]*scopolamine.* Labelled material (100 μc, 0.2 mg/kg) was injected s.c. to mice. They were decapitated at various time intervals and their brains quickly removed (within 2 min) and washed for 30 sec in saline. Each brain was placed in a Potter–Elvehjem glass homogenizer containing 5 ml distilled water and fitted with a teflon pestle. 0.2 ml portions of the homogenate were assayed for radioactivity in a standard mixture containing 0.2 ml distilled water and 3 ml of Insta-gel (Packard). Four mice were used for each time interval and the results expressed as the average of the four.

Binding experiments in vitro. A full description of the procedure for determining the affinity constants

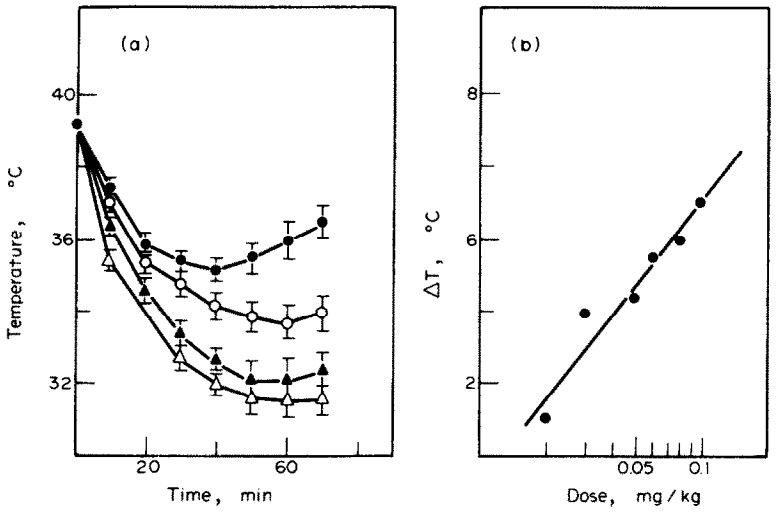

Fig. 1. (A) Time profile of the dose-dependent rectal temperature after 0.1 ml s.c. injection of oxotremorine to mice. Each curve describes a successive recording of the same group of mice, at a given dose. Values shown are the mean ± S.E. obtained in a group of 5 mice. Doses used: 0.03 mg/kg (●), 0.06 mg/kg (○), 0.1 mg/kg (▲) and 0.3 mg/kg (△). (B) Dose–response curve for the hypothermic activity of oxotremorine. Each point represents the maximal effect for each dose taken from A.

of the anticholinergic drugs towards the "muscarinic sites" in a preparation from mice brain homogenate is given elsewhere [15]. The preparation is a 1000 g supernatant of 10% brain homogenate in 0.32 M sucrose. A 0.05 ml portion was incubated for 30 min at 25° in a total volume of 2 ml of modified Krebs solution (118 mM NaCl, 4.69 mM KCl, 1.9 mM $CaCl_2$, 0.54 mM $MgCl_2$, 1.0 mM NaH_2PO_4, 11.1 mM glucose and 25 mM Tris–HCl, pH 7.4). The incubation mixture included [^{3}H]-*N*-methyl-4-piperidyl benzilate (NMPB) specific activity 6 Ci/m-mole or [^{3}H]NMPB and various concentrations of the ligand tested, as specified in each experiment. The incubation was terminated by the addition of ice-cold Krebs solution and filtration with GF/C filters. The filters were washed three times using the same Krebs solution and immersed in 5 ml Insta-gel (Packard) in plastic vials. Thirty min later the radioactivity was determined using liquid-scintillation counter (Packard) with a 33 per cent efficiency. The affinity constant of the drug towards the muscarinic receptor was calculated by plotting $1/RL^*$ (RL^* = the concentration of [^{3}H]NMPB-receptor complex) versus the drug's concentration. From the slope of the straight line obtained, the affinity constant was calculated.

[^{3}*H*]*Scopolamine.* Scopolamine specific activity 1.75 Ci/m-mole, was labelled by tritium exchange in the Nuclear Research Center, Negev, Israel.

RESULTS

The antimuscarinic potencies of three antidepressive drugs were evaluated in two central system effects (hypothermia, tremor) and in one peripheral system effect (mydriasis), as well as by using two *in vitro* preparations—muscarinic receptor and acetylcholinesterase, both from mouse brain whole homogenate.

Antihypothermic and antitremorgenic activity. The antihypothermic and antitremorgenic potencies of the tested drugs were determined by measuring their effects against these states induced by oxotremorine. Both the degree and the duration of the hypothermia induced in mice by s.c. administration of oxotremorine were found to be dose-dependent (Fig. 1). The oxotremorine was tested in a dose range of

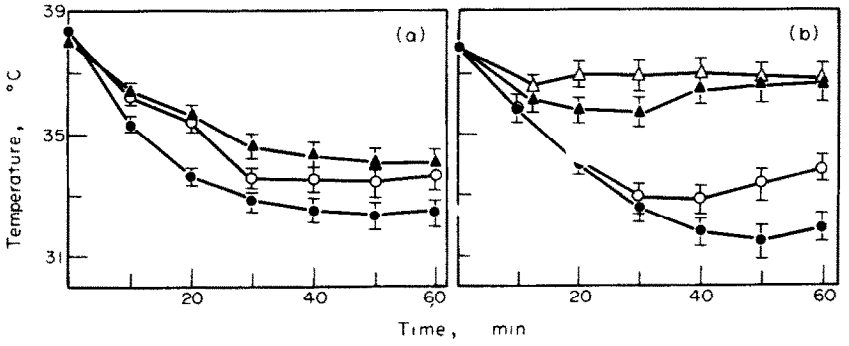

Fig. 2. Time profiles of the dose dependent rectal temperature after 0.1 mg/kg s.c. administration of oxotremorine following prior injection of imipramine or scopolamine. (a) 20 min after imipramine: 2 mg/kg (○). 10 mg/kg (▲) and control (●). (b) Simultaneously with scopolamine: 0.025 mg/kg (○), 0.05 mg/kg (▲), 0.1 mg/kg (△) and control (●). Each value represents the mean ± S.E. obtained in a group of 5 mice.

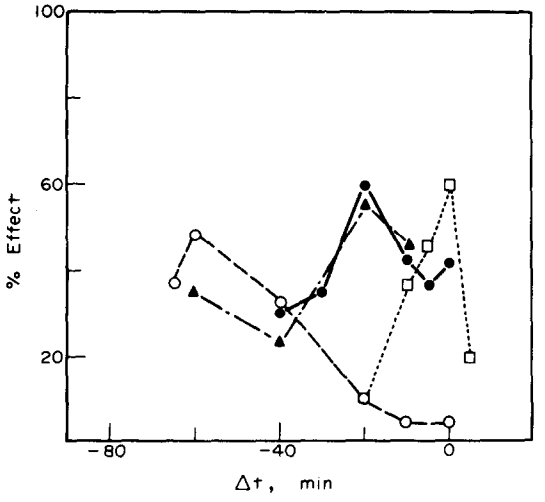

Fig. 3. The effect of the time interval between antidepressant and oxotremorine injection on the maximum antihypothermic effect. The antihypothermic drugs were injected s.c. (0.1 ml) at various times before oxotremorine (0.1 mg/kg) s.c. (0.1 ml) injection. Three experiments, 5 mice each, were performed for each time interval. The points are the means of the results (S.E. ± 7%): nortriptyline (5 mg/kg) (●), amitriptyline (2 mg/kg) (○), imipramine (5 mg/kg) (▲), scopolamine (0.1 mg/kg) (□).

0.03–0.3 mg/kg. By successive measurement of the hypothermic effect, the peak effect could be determined for each dose, and dose–response curves could be obtained. These curves were linear in the range of 0.03–0.1 mg/kg; the upper value was used for the study of the antihypothermic activity of the tricyclic antidepressants.

The time course of the hypothermia induced by 0.1 mg/kg oxotremorine administered 20 min after various doses of imipramine is shown in Fig. 2a. Scopolamine·HBr was used as a reference compound because it is considered an antimuscarinic drug with a profound central anticholinergic potency. As shown in Fig. 2b, simultaneous s.c. injection of 0.1 mg/kg oxotremorine and 0.025–0.1 mg/kg scopolamine either completely blocked the hypothermic effect of oxotremorine or partly antagonized its development.

Inch *et al.* [16] found the time profile of the antimuscarinic activity of atropine-like drugs to be linearly dependent on the minus logarithmus of their affinity constant to the muscarinic receptor. Thus, they emphasized the need to determine the time of the *in vivo* peak effect as a prerequisite to evaluating the relative anticholinergic potency in a given set of drugs. Indeed, determination of the relative antimuscarinic potency of a given set of drugs at a fixed time interval leads to completely different results. There-

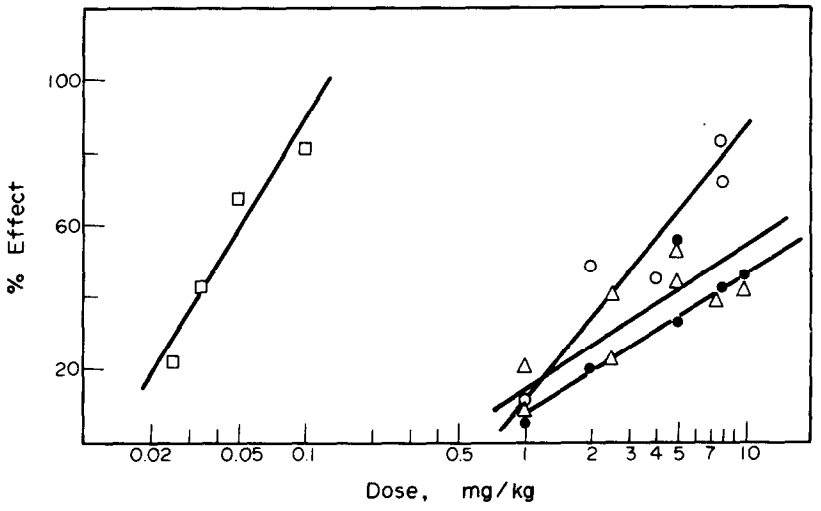

Fig. 4. Dose–response curves for antihypothermic activity of antidepressant drugs. The maximum values of the hypothermia (taken from Figures like Fig. 1) are represented. Amitriptyline (○), nortriptyline (△), imipramine (●), scopolamine (□).

Table 1. A summary of pheripheral and central anticholinergic potency of some tricyclic antidepressants

Drug	ED_{50}mg/kg*	ED_{50}mg/kg†	ED_{50}mg/kg‡	$K(M)^{-1}$§	$K(M)^{-1}$¶
Amitriptyline	3	4.5	6	3×10^7	3.2×10^7 [7, 8]
Nortriptyline	10	19	30	4.5×10^6	5.0×10^6 [7, 8]
Imipramine	8	7.5	23	5×10^6	7.1×10^6 [8]
Scopolamine	0.1	0.07	0.009	2.5×10^9	3×10^9

* ED_{50} for the antagonism of the hypothermic effect caused by oxotremorine (Figs 2, 4).
† ED_{50} for the antagonism of the tremorgenic effect caused by oxotremorine (Fig. 8).
‡ ED_{50} for the mydriatic effect.
§ Mouse brain homogenate.
¶ Guinea pig ileum preparation.

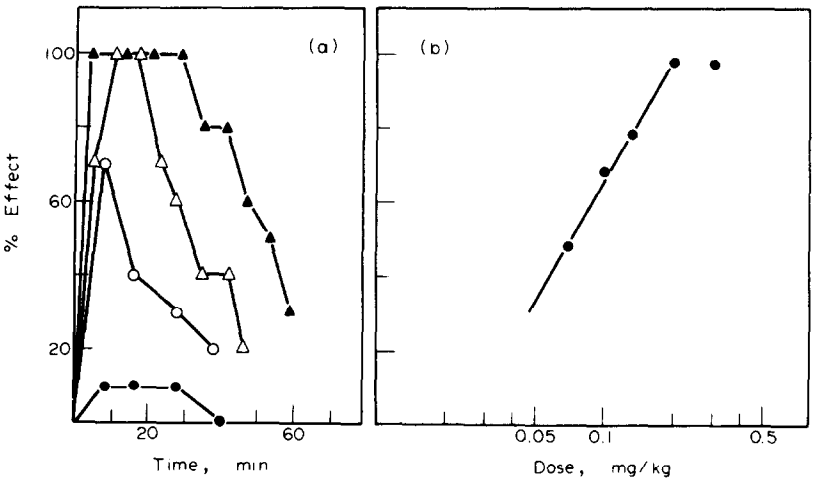

Fig. 5. (a) Time profile of the dose-dependent tremor induced in mice by 0.1 ml s.c. injection of oxotremorine, at the following doses. 0.05 mg/kg (●), 0.10 mg/kg (○), 0.20 mg/kg (△), 0.30 mg/kg (▲). See Methods for further details. (b) Dose-response curve for tremorogenic effect. Each point represents the maximal effect for each dose taken from (a).

fore, we measured the effect of the time interval between the administration of the antidepressants and oxotremorine on the magnitude of the antihypothermic effect (Fig. 3). The maximum antagonistic activity of nortriptyline, amitriptyline, imipramine and scopolamine developed at different rates, reaching maximum value at 20, 60, 20 and 0 min before oxotremorine administration, respectively. On the basis of these results, the dose–response studies of the antihypothermic activity of the four drugs were carried out at a different time schedule for each (Fig. 2). From these graphs a dose–response curve for the antihypothermic activity of each drug was constructed (Fig. 4). The ED_{50} values were calculated from these graphs and are presented in Table 1.

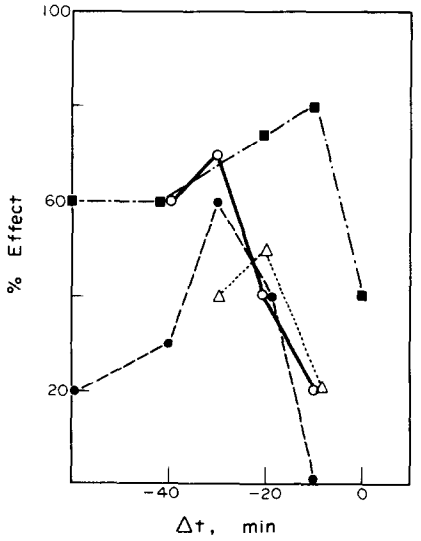

Fig. 6. The effect of the time interval between oxotremorine and antidepressant drugs administration on the maximum antitremorgenic effect. The antitremorgenic drugs were injected s.c. (0.1 ml) at various times before s.c. (0.1 ml) injection of oxotremorine (0.2 mg/kg). Three experiments, 10 mice each, were performed for each time interval. The points are the means of the results (S.E. ± 5%): amitriptyline (6 mg/kg) (○), nortriptyline (14 mg/kg) (●), imipramine (5 mg/kg) (△), scopolamine (0.08 mg/kg) (■).

Table 2. Optimal time intervals for the antagonistic activity of tricyclic antidepressants to hypothermia and tremor*

Drug (s.c.)	Optimal time interval (min) Antihypothermia†	Antitremor‡
Nortriptyline	−20	−30
Amitriptyline	−60	−30
Imipramine	−20	−20
Scopolamine	0	−10

* The data are taken from Figs 3, 6. Each value represents the results from two separate groups of 10 animals each.

† Hypothermia was induced by s.c. administration of 0.1 mg/kg oxotremorine.

‡ Tremor was induced by s.c. administration of 0.2 mg/kg oxotremorine. The presence of the effect was determined after 10 min.

In addition to its hypothermic effect, oxotremorine is believed to induce a tremorgenic response of central origin [10, 17]. Indeed, Inch *et al.*, used these central effects to evaluate the relative antimuscarinic potencies of a whole set of atropine-like drugs [16]. However, no details could be found in the literature for the dose-dependent time profile of oxotremorine activity. Thus, in order to choose both the proper dose and the correct time schedule of oxotremorine activity for evaluating the antitremorgenic potency of the antidepressants, the time course for its tremorgenic activity was determined in the dose range of 0.05–0.3 mg/kg (Fig. 5a). Both the percentage of the effect and the duration of the response were dose-dependent, and dose–response curves were obtained (Fig. 5b). The minimum dose which still caused a full effect was selected as the standard dose for evaluating the antidepressants.

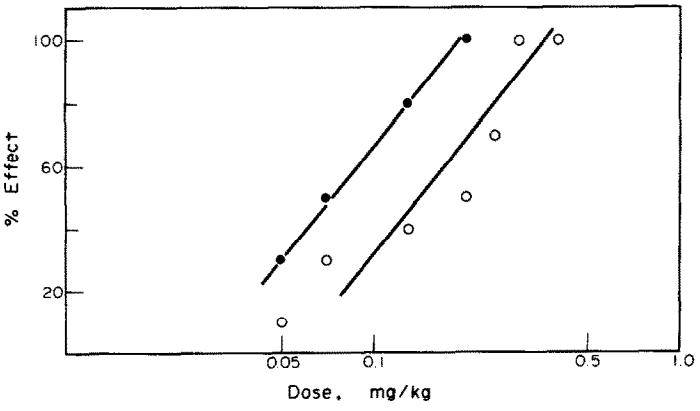

Fig. 7. Dose–response curves for the tremor induced by s.c. administration of 0.2 mg/kg oxotremorine with (○) or without (●) prior injection (30 min) of 4.5 mg/kg amitriptyline.

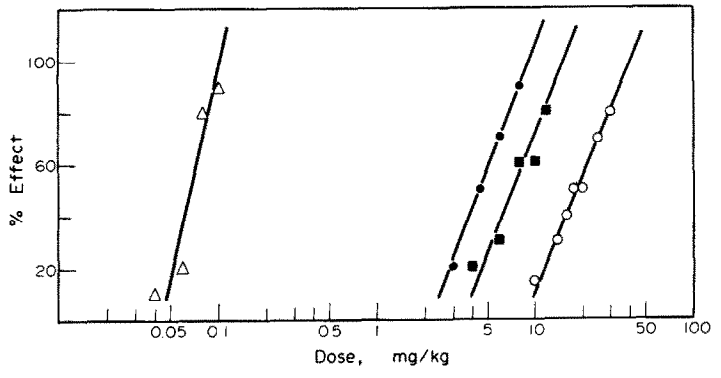

Fig. 8. Dose–response curves for antitremorgenic drugs. Drugs were injected s.c. at optimum times before oxotremorine (see Fig. 6). Tremor was assessed visually 15 min after the oxotremorine injection (see Methods for further details). Scopolamine (10 min before oxotremorine, △), amitriptyline (30 min, ●), nortriptyline (30 min, ○), imipramine (20 min, ■).

The time profiles for the antitremorgenic activity of the drugs had to be determined since they were not necessarily the same as for their antihypothermic activity. The results (Fig. 6, Table 2) showed that the maximal antitremorgenic activity of nortriptyline, amitriptyline, imipramine and scopolamine was achieved at different time intervals: 30, 30, 20 and 10 min, respectively, before the injection of 0.2 mg/kg oxotremorine. These values are clearly different from those found for the antihypothermic activity. Interestingly, scopolamine·HBr was found in both cases to exert its antimuscarinic activity at the shortest time interval.

The time course of the tremor induced by oxotremorine after a prior injection of various doses of the drugs tested reflected a competition between the two kinds of compounds: the increase in the dose of the antidepressant led to a parallel decrease in both the percentage and duration of the developing tremor (see Fig. 7, amitriptyline). Moreover, the dose-response curves obtained for all four drugs tested were parallel and the ED_{50} values could be interpolated (Table 1, Fig. 8).

Mydriatic activity. The antimuscarinic activity of the drugs was clearly manifested in their mydriatic activity. Indeed, this peripheral response is frequently exploited by investigators as a "direct" method for evaluating peripheral antimuscarinic potency, since only one drug has to be systematically administered to the subjects. Although the mydriatic activity is a measure of overall competition between a muscarinic agonist (endogenous acetylcholine) and the tested drug, it has the advantage of being measured quantitatively at a response range of 20–80%, thereby en-

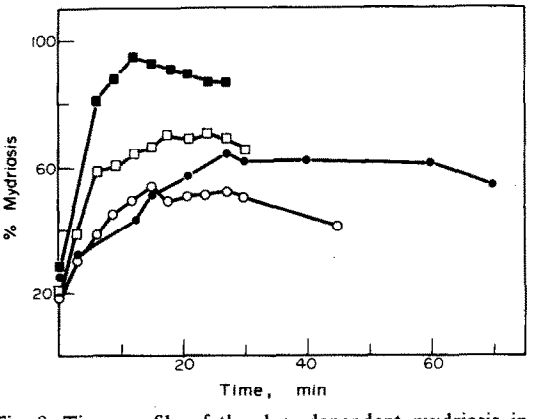

Fig. 9. Time profile of the dose-dependent mydriasis induced in mice after s.c. administration of amitriptyline and scopolamine. Each point represents the mean response induced in 6 mice, at the following doses: amitriptyline 5 mg/kg (○) and 20 mg/kg (□), scopolamine 0.01 mg/kg (●) and 0.1 mg/kg (■).

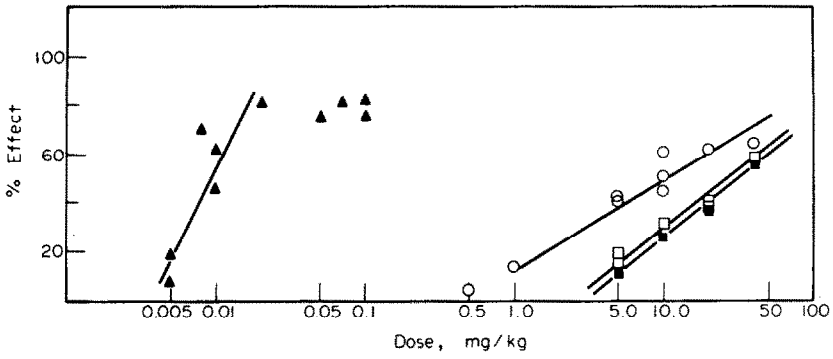

Fig. 10. Dose–response curves for the mydriatic activity of tricyclic antidepressants: nortriptyline (■), imipramine (□), amitriptyline (○) and scopolamine (▲).

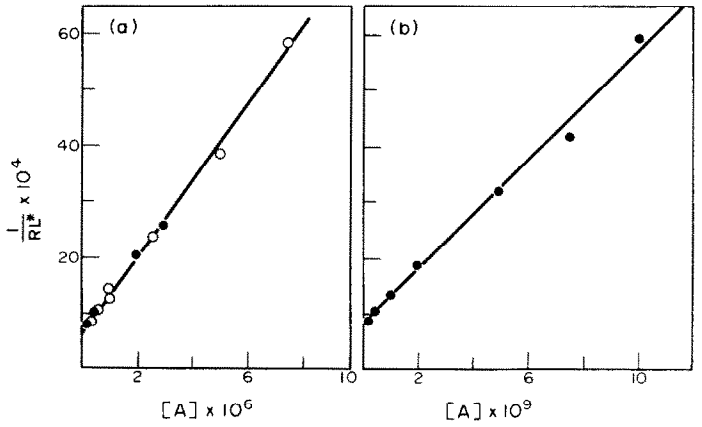

Fig. 11. Competition experiments using [^{3}H]NMPB and anticholinergic drugs. (a) Competition of [^{3}H]NMPB with imipramine (○) with nortriptyline (●). (b) Competition of [^{3}H]NMPB with scopolamine.

Table 3. Anti-acetylcholinesterase activity of tricyclic antidepressants

Drug	K_i (M)
Amitriptyline	3.6×10^{-4}
Nortriptyline	3.4×10^{-4}
Imipramine	3.4×10^{-4}

abling a finer evaluation of the drugs relative potencies. The dose dependent time profiles for the mydriatic activity of amitriptyline, together with that of scopolamine·HBr, are presented in Fig. 9. The dose–response curves are shown in Fig. 10.

Binding experiments. Brimblecombe *et al.* [8] showed the tricyclic antidepressants to have a considerable affinity to peripheral muscarinic systems. In view of Beld *et al.*'s finding [18] of a good correlation between peripheral antimuscarinic potency and the binding constant towards the central muscarinic receptor for atropine, we decided to determine the affinity of our drugs towards the muscarinic receptor from the mouse brain.

The binding constant towards the muscarinic receptor from the mice whole brain homogenate was determined *in vitro* (Fig. 11, see also Methods). Plotting $1/RL^*$ values against the concentration of the tested ligand gave straight lines from which both the binding constant and the number of the total muscarinic receptors could be calculated. The binding constants to the central muscarinic receptor obtained by this method are summarized in Table 1.

Acetylcholinesterase inhibition. Many central-acting drugs were found to exert multiple biochemical interactions rather than one specific biochemical activity. For example, the analgetic drug morphine and many of its congeners are known to bind to cholinesterase [19]. Likewise, phencyclidine and its derivatives were found to bind to both the central cholinesterase and the muscarinic receptor [20, 21]. Although they are amines with a high degree of protonation at physiological pH, the antidepressants investigated

Table 4. * Intratomic distances in imipramine molecule

	C_5	C_6	C_7	N_8
C_1	4.0	4.9	5.6	6.8
C_2	2.5	3.5	4.4	5.6
C_3	2.2	3.3	4.5	5.8

* Lengths in A^0 of intratomic distances in imipramine according to the crystal structure found by M. L. Post [29].

here were found to bind only loosely to acetylcholinesterase from the mouse brain (Table 3). This low affinity, which is clearly demonstrated by the relatively high K_i values, resembles that of choline. Similar results were reported by Ho *et al.* [22] and by Caratsch *et al.* [23]. Thus, as far as the central cholinergic system is concerned, the three antidepressants interact with the muscarinic receptor *in vitro* with higher affinity of two orders of magnitude relative to their affinity to the central acetylcholinesterase from the mouse brain.

DISCUSSION

Determination of the relative affinity of the tricyclic antidepressants towards the central muscarinic nervous system requires knowledge of their relative anticholinergic potency *in vivo*. Unfortunately, the quantitative data available on their antimuscarinic activity *in vivo* are derived from a variety of experimental procedures, preventing a comprehensive comparison. In order to use a standard *in vivo* procedure we exploited one of the criteria formulated by Inch *et al.* [16] which, if fulfilled, enables one to overcome many of the difficulties found in this kind of experiment. This criterion requires plotting the whole time course of the drug activity *in vivo*, and determination of the peak response as a means of comparing the relative potencies. Indeed, the time intervals to the peak antimuscarinic activity were specific for both the drug and the effect measured. As shown in Table 2, these intervals range from 0 to 60 min for the antitremorgenic and antihypothermic effects. Moreover, both scopolamine and amitriptyline developed their antitremorgenic and antihypothermic effects at a different rate. As a consequence, a specific time schedule was adopted for each drug and for each antimuscarinic effect.

One way of comparing the relative anticholinergic potencies of the drugs is to calculate their ED_{50} values from the dose-response curves (Table 1, Figs 4, 8). Our values were lower than those reported previously [8, 24]. This discrepancy could result from a number of factors, including a different route of drug administration, an arbitrary time interval selected between the times of drug and oxotremorine administration, and varying dose ratios of the two compounds. It is our feeling that the specific conditions laid down in our experiments for determining the relative *in vivo* antimuscarinic potency is a more correct approach, although much more time consuming. Interestingly, the relative antimuscarinic potencies found for the three antidepressants is maintained in the two central effects. Thus, nortriptyline is the least active, while amitriptyline is the most potent drug. Scopolamine, the reference compound, has a ten-fold higher potency than amitriptyline (Table 1).

According to Inch *et al.* [25], there is a linear correlation, which is not yet understood, between the affinity constant of a given drug towards the muscarinic receptor and the time profile of its biological effects. A longer onset time and a more prolonged duration was found for the antimuscarinic drug with a greater affinity towards the receptor. It was expected that by using equipotent doses (ED_{50} values), the tricyclic antidepressants would manifest their anticholinergic effects more quickly than scopolamine, because their affinity constants reported by Brimble-

Table 5. A comparison between the intratomic distances in benactyzine in the crystal conformation and in the superimposed conformation*

	C_4	C_5	O_1	O_2	C_6	C_7	N_8
C_1	3.9	4.7	4.0	5.2 (5.4)	5.9	6.1 (6.5)	6.8 (7.2)
C_2	2.5	3.5	2.7	4.0 (4.0)	4.5	4.9 (5.2)	5.6 (6.1)
C_3	1.6	2.5	2.4	3.2 (3.7)	4.0	4.8 (5.3)	5.8 (6.3)

* Lengths in A^0 of intratomic distances in benactyzine calculated from superimposition of this molecule with imipramine using Dreiding Models. The figure in brackets is the length of intratomic distance in benactyzine according to A. Meyerhoffer [28].

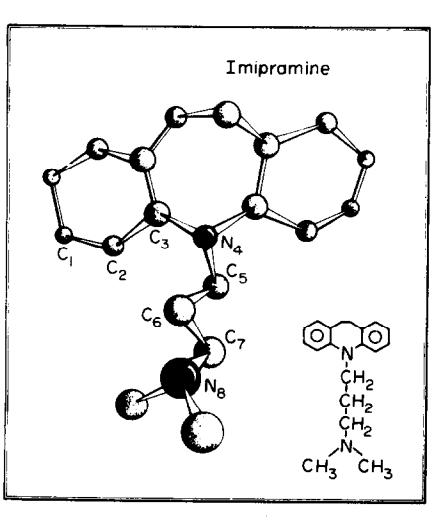

Fig. 12. Molecular model of imipramine as found in the crystal structure [28].

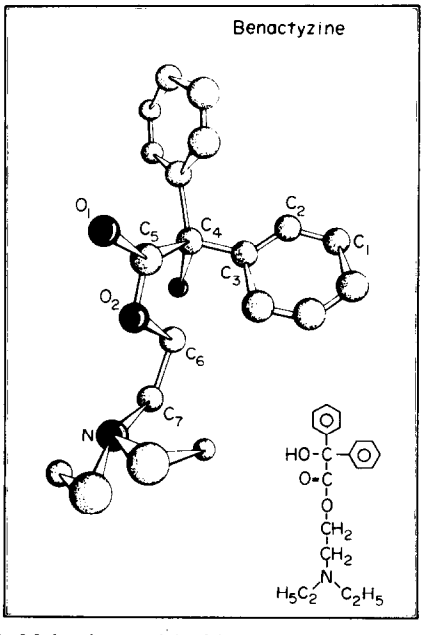

Fig. 13. Molecular model of benactyzine as found in the crystal structure [29].

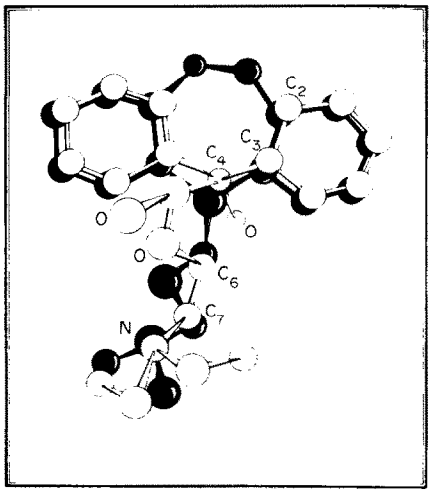

Fig. 14. Superposition of imipramine and benactyzine molecules.

combe *et al.* [8] for peripheral muscarinic system are lower by 2.5 orders of magnitude (Table 1). Since the same values were found here for the binding constants towards the central muscarinic receptor, and because the onset times of the antidepressants are longer than those of scopolamine (Table 2), it seems that Inch's theory is valid mainly for pure antimuscarinic drugs. Indeed, it is possible that these compounds are not pure antimuscarinic drugs, mediating their central effects via mixed neuromechanisms. However, the purely competitive nature of their antimuscarinic effect both *in vivo* (Figs 4, 7, 8) and *in vitro* (Fig. 11) tempt one to conclude that their antitremorgenic and antihypothermic effects are also mediated by cholinergic mechanisms.

In order to further investigate the long onset time of the drugs' anticholinergic effects, a peripheral antimuscarinic response was selected and its *in vivo* time course followed. As illustrated in Fig. 9, the mydriatic activity of the tricyclic antidepressants, induced by systemic administration, has again a longer onset time than that of scopolamine. It will be of interest to follow the kinetics of their interaction with the central muscarinic receptor *in vitro*, as well as the time course of their peripheral antimuscarinic effects in, e.g., isolated organs, in order to gain some biochemical interpretation to the *in vivo* time profile.

The binding constants towards the central muscarinic receptor, determined *in vitro*, could be considered the lower limit for the drug concentration needed to block half of the available muscarinic sites. However, because an antimuscarinic drug is expected to compete *in vivo* both with endogenous (Ach) and exogenous (oxotremorine) muscarinic agonists, one assumes that a much higher concentration has to be built up in the CNS in order to induce measurable central antimuscarinic activity. One possible approach for determining the concentration of a drug in an organ *in vivo* is to follow its uptake, using labelled material. Using [^{3}H]scopolamine it was found that the peak concentration of [^{3}H]scopolamine in the mouse brain is 4×10^{-7} M/kg tissue, which is higher by two orders of magnitude than its binding constant. In a different study using various labelled psychotropic drugs we found that the amount generally taken up by the mouse brain *in vivo* is around 1 per cent of the dose injected. Thus, it is reasonable to assume that the concentration of the tricyclic antidepressants in the brain will be around 10^{-5} M/kg tissue, at the ED_{50} value, which exceeds by at least one order of magnitude their binding constant. Thus, it seems justifiable to compare the binding constants found *in vitro* with the pharmacological values determined *in vivo*, as shown in Table 1. In a recently published report, Sayers *et al.* [24] determined the IC_{50} values for [^{3}H]quinuclidinyl benzilate displacement by several antidepressant drugs *in vitro*. This approach allows only a comparative study of the drugs selected towards the muscarinic receptor since their values depend on both the concentration and the affinity of the labelled drug used. On the other hand, determination of affinity constants of antimuscarinic drugs towards the receptor, as done in this work, permits a comparison with results obtained in other muscarinic systems.

A competition between two compounds on the same biological site is believed to indicate a possible common molecular structure. Thus, among agents manifesting antimuscarinic activity, three necessary chemical groups have to coexist—a cationic group, an aromatic group and a hydroxylic group [26]. These reactive regions were found by analysis of X-ray diffraction pattern to be spatially similarly arranged. In our work, a putative antimuscarinic drug, suggested by Ariens *et al.* [26 and references sited therein] to incorporate the necessary chemical elements for antimuscarinic activity, was found by examining molecular models to fit the rules formulated by Guy *et al.* [27] and Meyerhoffer [28]. The antimuscarinic character of the tricyclic antidepresants may also reflect a molecular resemblance to the identical "antimuscarinic pharmacophore". The molecular structure of imipramine, in one of the preferred conformations found in the crystal state [29], is shown in Fig. 12, while the intratomic distances are summarized in Table 4. The molecular structure of benactyzine, as found in the crystal [30], is shown in Fig. 13. This drug represents an aminoester of benzilic acid, known to have high antimuscarinic activity [28].

In order to verify the possibility of imipramine mimicking the spatial arrangement of benactyzine, we examined both CPK and Dreiding molecular models. Both aromatic rings of benactyzine could be superimposed on those of imipramine without steric hindrance as judged by CPK molecular models. The superposition of the rest of the benactyzine molecule on that of imipramine slightly changes the dihedral angles found in the crystal, but the imipramine still mimics the allowed conformation as found by CPK molecular models (Table 5, Fig. 14).

Although it is not yet clear whether the antimuscarinic component of the tricyclic antidepressants contributes to their antidepressive activity, it is clear that such interactions must be taken into consideration when designing new analogs.

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