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IN VIVO AND *IN VITRO* STUDIES ON THE ANTIMUSCARINIC ACTIVITY OF SOME AMINO ESTERS OF BENZILIC ACID

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Abstract—The cholinergic antimuscarinic properties of some aliphatic and heterocyclic aminoesters of benzilic acid were evaluated in the CNS as well as in peripheral organs. Antagonism to hypothermia and to forced motor activity disturbances, both centrally mediated and induced in mice by oxotremorine, was kinetically determined *in vivo*. The antiacetylcholine activity of these compounds on the isolated guinea pig ileum, and their affinity for muscarinic high-affinity binding-sites in mouse brain homogenate were determined and correlated *in vitro*. 3-Quinuclidinyl benzilate (QNB) was found to be the most potent drug *in vivo* as well as *in vitro*, while dimethylaminoethyl benzilate was the least active. Atropine was as potent as scopolamine in the competition experiments *in vitro* but ten times less active in the two *in vivo* tests. Rate constants for the onset (k_{on}) and offset (k_{off}) of the antimuscarinic activity determined on the isolateed ileum, were found to increase and decrease, respectively, with the increase in the affinity of the drugs for the muscarinic binding-sites. The relationship between the molecular structure and the properties of these compounds is discussed in terms of the factors contributing to the observed rate constants of antimuscarinic activity and of the reasons for the decrease in the apparent potency of atropine *in vivo*.

The aminoesters of benzilic acid are among the most potent antimuscarinic as well as psychotropic drugs known [1–6]. Two of these compounds—benziloxycholine mustard and 3-quinuclidinyl benzilate have been extensively used to study cholinergic muscarinic interactions, in both *in vivo* and *in vitro* experiments [7–11].

Recently, procedures have been developed for measuring the binding of muscarinic ligands to muscarinic binding-sites from mammalian brains *in vitro* [8, 12–13]. In this work the *in vitro* experiments using [^{3}H]*N*-methyl-4-piperidyl benzilate ([^{3}H]4NMPB) were combined with simple and rapid methods for estimating antimuscarinic potencies *in vivo* in order to characterize the antimuscarinic properties and the underlying structure-function relationships of some aminoesters of benzilic acid.

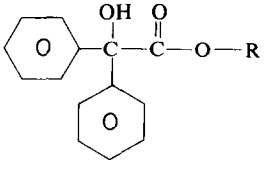
MATERIALS AND METHODS

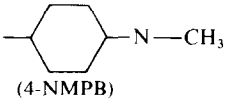
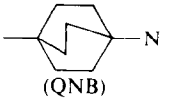
Drugs. The following compounds were dissolved in physiological saline solution: oxotremorine (Aldrich), (−) scopolamine. HCl (Plantex, Israel), atropine sulfate hydrate (Sigma), acetylcholine perchlorate (BDH). All other drugs used were of analytical purity. Benzilic acid esters were prepared according to Biel *et al.* [14]. Some of their chemophysical parameters are summarized in Table 1.

[^{3}H]-scopolamine (1.75 Ci/m-mole), [^{3}H]-atropine (5.3 Ci/m-mole) and [^{3}H]*N*-methyl-4-piperidyl benzilate (6 Ci/m-mole) generally labelled were prepared as described by Kloog and Sokolovsky [15].

Animals. Adult male mice, weighing 18–24 g ICR strain, were used throughout. Animals were allowed

Table 1. Physiochemical properties of several aminoesters of benzilic acid



—R	H Cl Salt m.p.*	T.L.C. System (R_f)†
—CH_2 CH_2 $N(CH_3)_2$ (DMAEB)	224	A 0.6
—CH_2 CH_2 $N(C_2H_5)_2$ (DEAEB)	164	B 0.3
 (4-NMPB)	203	A 0.7
 (QNB)	242	C 0.6

* Not corrected.

† A—Silica (Woelm) with water-methanol-glacial acetic acid (1:10:10). B—Silica (Woelm) with ethanol. C—Silica (Woelm) with chloroform-acetone-diethylamine (1:4:5).

free access to food and drinking water until the commencement of an experiment. Housing and laboratory temperatures were maintained at 23 ± 0.5°. The drugs were injected subcutaneously (s.c.) to the mice, in volumes which did not exceed 0.1 ml/animal.

Measurement of hypothermia. Rectal temperature, measured by a telethermometer (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 antimuscarinic drug. The percentage of the effect was defined as $\frac{T_1 - T_2}{T_1} \times 100$, where T_1 was the maximal change in temperature after oxotremorine injection and T_2 was the maximal change in temperature after the consecutive injections of the antimuscarinic drug and oxotremorine at an optimal time interval (*vide infra*).

Rotarod test. Mice were placed on a rod, 32 mm in diameter, rotating at 16.5 rpm. Sideward movements on the rod were limited by circular discs set 19 cm apart. The mice were trained until able to stay on the rotarod for at least 120 sec and then they were injected s.c. with 1.2 mg/kg of oxotremorine. At this dose none of the tested animals could cling to the rod for at least 90 sec. This 100 per cent falling effect, which was tested every 6 min for 120 sec lasted over 40 min, and 60 additional min were needed for full recovery (Fig. 1). Injection of 1.2 mg/kg oxotremorine every 30 min, five times, prolonged the duration of the peak effect, but did not change the rate of recovery (Fig. 1). A control group of mice injected similarly but tested beginning 20 min after the last injection also recovered at the same rate (Fig. 1), thus eliminating the possibility of "learning" during the test.

Ten min after the first injection of oxotremorine, the treated mice were injected with the antimuscarinic drug tested, and the kinetics of the recovery was followed as well as the kinetics of the return to the initial conditions. The dose-dependent time profiles of this antioxotremorine activity (Fig. 2), were used to estimate the central antimuscarinic potency of the drugs in this study, by plotting the value of the peak effects obtained at each dose against the logarithm of the dose. From these dose-response curves ED_{50} values were calculated.

Brain uptake of labelled drug. Labelled material (100 μc) was injected s.c. to mice at doses around the ED_{50} values as determined by the two *in vivo* tests. The animals were decapitated at various intervals and their brains removed within 2 min and washed for 30 sec in saline. Each brain was placed in a glass homogenizer containing 5 ml distilled water and fitted with a Teflon pestle. 0.2 ml portions of the homogenates were assayed for radioactivity in a standard mixture containing 0.2 ml distilled water and 3 ml Insta-gel (Packard) using liquid scintillation counter (Packard) with a 33 per cent efficiency.

Binding experiments in vitro. The affinity constants of the tested drugs towards the muscarinic receptor were determined by competition with [^{3}H]4NMPB. The interaction of [^{3}H]4NMPB with the muscarinic binding-sites in mouse brain homogenate and the theoretical basis for treating the experimental data is given elsewhere [15]. The preparation is a 1000 *g* supernatant of 10% (w/v) 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 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]4NMPB alone or with various concentrations of the ligands and was tested as specified in each experiment. Every determination of binding was performed in triplicate. The incubation was terminated by the addition of ice-cold Krebs solution and by filtration with GF/C filters (Whatman), positioned over a vacuum. The filters were washed three

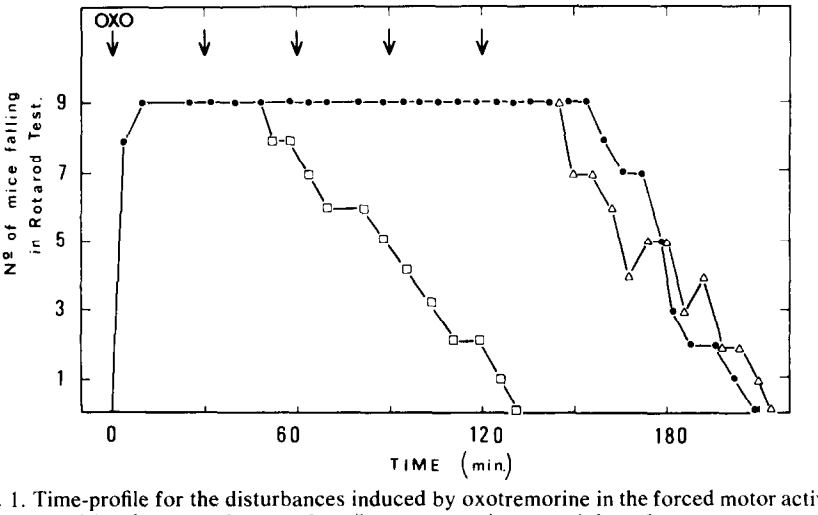


Fig. 1. Time-profile for the disturbances induced by oxotremorine in the forced motor activity of mice as measured by the rotarod test. 1.2 mg/kg oxotremorine were injected s.c. to a group of nine mice trained previously to cling to the rotarod. Their ability to cling to the rod was tested every 6 min during 120-sec trials and the effect of oxotremorine is described as the number of mice unable to cling to the rotarod. Three groups were used: (1) was injected five times at 30-min intervals and tested every 6 min (●), (2) was similarly injected but tested starting 20 min after the last injection (△) and (3) was injected only once (□).

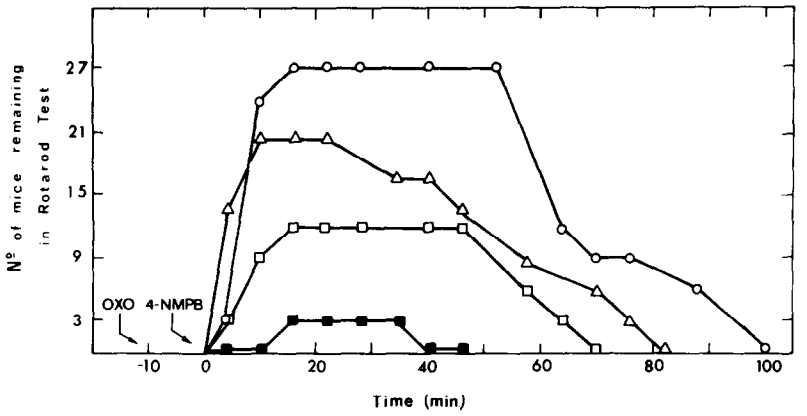


Fig. 2. Dose-dependent time profile for the antioxotremorine activity of 4NMPB as determined by the rotarod test. Ten min after the first injection of oxotremorine, various doses of 4NMPB were s.c. administered using twenty-seven mice for each dose (three groups of nine mice each). The number of mice able to cling to the rotarod is described as a function of time. Oxotremorine was administered (1.2 mg/kg) every 30 min. Doses of 4NMPB used: 0.1 mg/kg (○). 0.07 mg/kg (△), 0.03 mg/kg (□) and 0.01 mg/kg (■). The points plotted are the mean of the results (S.E.M. ± 10%).

times under vacuum using the same Krebs solution, and immersed in 5 ml Insta-gel (Packard) in plastic vials. Thirty minutes later the radioactivity was determined using a scintillation counter (Packard) with a 33 per cent efficiency. The dissociation constants of the drugs from the muscarinic binding-sites were calculated by plotting $1/RL^*$ (RL^* = the concentration of the specific bound [^{3}H]4NMPB) vs the drug concentration. The dissociation constant was calculated from the slope of the line obtained. The specific binding is defined as the total binding of [^{3}H]4NMPB minus the nonspecific binding (in the presence of 5×10^{-5} M unlabelled 4NMPB). Non-specific binding did not exceed 3 per cent of the total binding at the [^{3}H]4NMPB concentration used.

Antagonism of acetylcholine induced contraction of the isolated guinea pig ileum. The cholinergic dissociation constants of the drugs were determined according to the Edinburgh staff using isolated smooth muscle from the guinea pig ileum [16]. Kinetic parameters for the anticholinergic response were estimated in this organ according to Paton [17].

RESULTS

The antioxotremorine potency of the drugs selected, as was measured by the forced motor activity test, was found to be both dose and time-dependent, as shown in Fig. 2 for 4NMPB. The dose-response relationships of these behavioral effects were estimated from the values of peak effects induced by each dose. Since these peak values were obtained at different intervals for each dose, a whole time-profile had to be determined. Dose-response curves composed from the peak values are shown in Fig. 3, and the ED_{50} values calculated from these curves are summarized in Table 2.

As shown in Table 2, the antioxotremorine

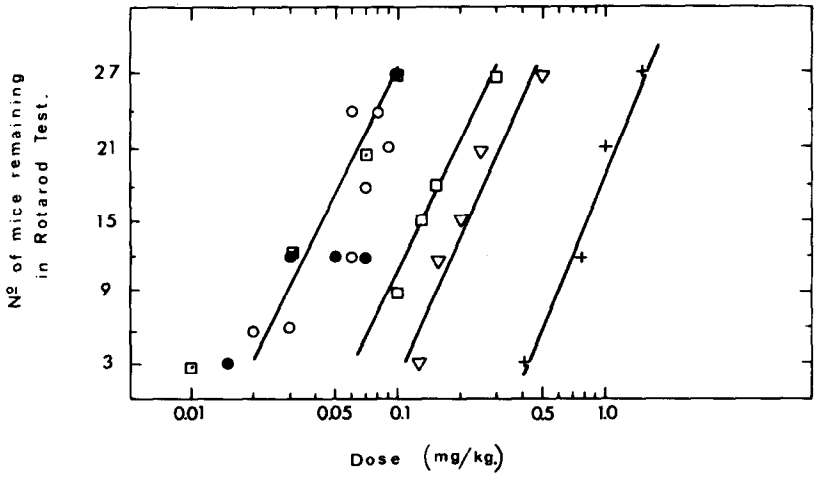


Fig. 3. Dose-response curves for the antioxotremorine activity of aminoesters of benzilic acid as determined by the rotarod test. Peak effects obtained for each dose were plotted vs the logarithm of the doses injected. Each point represents the results obtained from twenty-seven mice (three groups of nine mice each, S.E.M. ± 10%). The following drugs were tested: atropine (+), dimethylaminoethyl benzilate (▽), diethylaminoethyl benzilate (□), scopolamine (●), *N*-methyl-4-piperidyl benzilate (⊡) and 3-quinuclidinyl benzilate (○).

Table 2. Antioxotremorine activity of the aminoesters as measured by the rotarod test in mice

Drug	ED_{50} μmoles/kg	EPMR*	Duration,† min	Duration (min) / ED_{50} (μmoles/kg)
DMAEB	0.57	5.2	40 ± 10	70
DEAEB	0.33	3.0	60 ± 10	180
4NMPB	0.11	1.0	70 ± 10	640
QNB	0.11	1.0	> 4 h	> 3600
Atropine	1.07	9.7	65 ± 10	60
Scopolamine	0.11	1.0	90 ± 10	820

* Equipotent molar ratio relative to that of scopolamine.
† At the ED_{50} value. Data are expressed as the mean ± S. E. M.

potency of the drugs studied here spans over one order of magnitude. Two compounds were found to be equipotent to scopolamine, 4NMPB and the racemate of QNB whereas the two aliphatic aminoesters—dimethylaminoethyl benzilate (DMAEB) and diethylaminoethyl benzilate (DEAEB) have EPMR values of 5.2 and 3.0, respectively. The least active drug is atropine (EPMR = 9.7).

In addition to the ED_{50} values, the duration of the antioxotremorine activity could also be used for a comparative characterization of the antimuscarinic nature of the drugs. Since the duration is dose-dependent (Fig. 2), it has to be compared at equipotent doses of the drugs. In Table 2 the duration of the antioxotremorine activity at the ED_{50} doses are given. As shown in this Table, the more potent drugs exert their central effects for longer periods, although lower doses were used to elicit the same peak effects. This point can be quantified by relating the duration to the ED_{50} values: while atropine and DMAEB are the least potent and the most short-acting drugs (duration to ED_{50} ratios are 60 and 70, respectively), 3-quinuclidinyl benzilate is both the most potent and the longest acting compound (duration/ED_{50} > 3600).

The central origin of the antioxotremorine activity of the drugs in the rotarod test could be verified by using quaternary drugs. For example, scopolamine-butylbromide at doses up to 0.9 mg/kg (which is equivalent to 10 × ED_{50} of scopolamine · HBr) did not antagonize the oxotremorine effects.

The second effect used for estimating the relative central antimuscarinic potency of the drugs was their antagonism to the hypothermia induced in mice by oxotremorine. In this test the anticholinergic drug is administered before oxotremorine, therefore the optimal time interval needed for the development of maximal antagonistic effects had to be determined for each drug. As shown in Table 3, these time intervals differ markedly for the antimuscarinic drugs tested here. For example, QNB had to be administered 40 min before oxotremorine, while scopolamine caused maximal antihypothermic effect when injected to mice simultaneously with oxotremorine.

A representative time-profile for the hypothermia induced by 0.1 mg/kg oxotremorine (s.c.) in mice, with and without pre-injection of an anticholinergic drug is shown in Fig. 4. ED_{50} values calculated from dose-response curves which were composed from the peak antihypothermic effects (curves not shown) are summarized in Table 3. Again, QNB and 4NMPB were found to be the most potent drugs, while atropine is the least active. The EPMR values relative to scopolamine are 0.5, 0.5 and 12, respectively.

The competitive nature of the antihypothermic

Table 3. Antihypothermic activity of the aminoesters in mice

Drug	ED_{50} μmoles/kg	EPMR*	Optimal time interval, min†
DMAEB	1.07	13	0
DEAEB	0.50	6.2	0
4NMPB	0.04	0.5	0
QNB	0.04	0.5	−40
Atropine	0.96	12	−40
Scopolamine	0.08	1.00	0

* Equipotent molar ratio relative to that of scopolamine.
† Optimal time interval for injection of the anticholinergic drug relative to 0.1 mg/kg oxotremorine in order to get maximum effect.

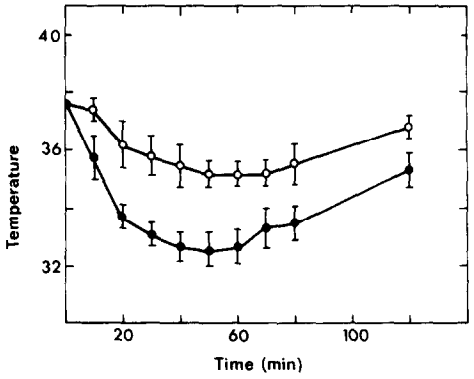


Fig. 4. Time-profile for the antihypothermic activity of diethylaminoethyl benzilate. Rectal temperature of mice was measured after 0.1 mg/kg s.c. administration of oxotremorine with (○) and without (●) prior injection of diethylaminoethyl benzilate (0.3 mg/kg). Each point represents the average temperature of five mice in two different experiments (S.E.M. ± 0.5°–1°).

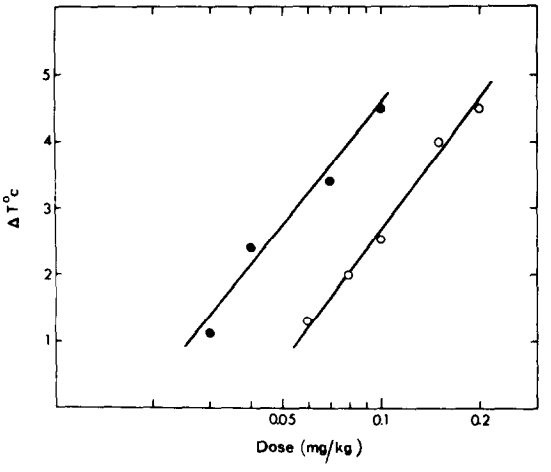


Fig. 5. Dose-response curves for the hypothermic effect induced in mice by oxotremorine, with (○) or without (●) simultaneous injection of scopolamine (0.03 mg/kg).

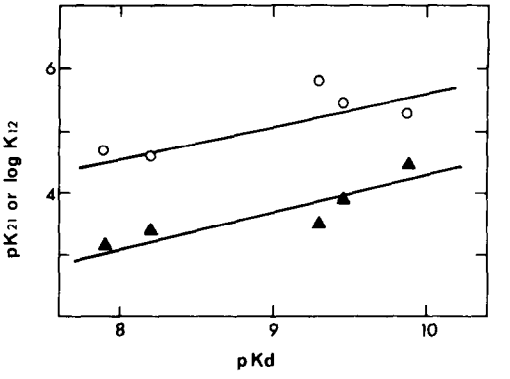


Fig. 6. The relation between the rate constants of the aminoesters and their dissociation constants as measureed in the guinea pig ileum. The log of K_{12} (○) or the −log of K_{21} (pK_{21}) (▲) are plotted verus −log K_d (pK_d). The data shown include all the drugs in Table 4 except for compound 4.

effect was verified by determination of dose-response curves to oxotremorine with and without preinjection of a constant dose of an antimuscarinic drug. As shown in Fig. 5, the dose-response curve of oxotremorine was found to be shifted in a parallel fashion relative to the control, towards higher doses of oxotremorine.

Evaluation of the antiacetylcholine potency of the drugs in the isolated guinea pig ileum (Table 4) showed that the K_d values span over two orders of magnitudes. For example, DMAEB with the lowest affinity towards this organ had $K_d = 1.3 \times 10^{-8}$ M and 4NMPB had $K_d = 1.3 \times 10^{-10}$ M. All the dose-response curves to ACh in the presence of all drugs except QNB, were shifted in a parallel manner relative to control.

The slope of the dose-response curve for the contractile response to ACh in the presence of QNB was found to be considerably lower than that of the control, and the dissociation constant (K_d) could not be calculated from this curve. It seems that unusual effects of ± QNB were also observed by Abramson *et al.* [18] who reported K_d values of several series of antimuscarinic drugs in the guinea pig ileum. In this report, the K_d value for QNB was only approximated—"10^{-10}–10^{-11}?".

Rate constants for the build-up and the decline of the antiacetylcholine activity of these drugs, determined by Patons' procedure [17], are summarized in Table 4, and a log–log plot of these and the dissociation constants is depicted in Fig 6. As shown in Table 4 and in Fig. 6 the "on" and the "off" rate constants (k_{12} and k_{21} values) increased and decreased, respectively, in parallel with the increase of the affinity of the drug towards the organ (i.e. high affinity correlates fully with a low k_{21} and a high k_{12}).

Representative linear curves obtained from competition experiments carried out *in vitro* on the muscarinic binding-sites from mouse brain homogenate are shown in Fig. 7 and the K_d values calculated from such graphs are given in Table 4. These values span over two orders of magnitude, and are quantitatively in a good agreement with those determined in the isolated guinea pig ileum. Thus, scopolamine, 4NMPB and QNB are equipotent, with $K_d = 5 \times 10^{-10}$ M, while DMAEB had the lowest affinity for the muscarinic binding-sites ($K_d = 2.7 \times 10^{-8}$ M).

The two structurally related reference compounds used, atropine and scopolamine, were found to exert almost equipotent antimuscarinic activity in both isolated smooth muscle and mouse brain homogenate preparations (Table 4), as shown also by Inch [6], Snyder [9], Baumgold *et al.* [11] and

Table 4. Dissociation and rate constants for the antiacetylcholine activity of some aminoesters of benzilic acid

Drug	K_{on} sec^{-1} M^{-1}	K_{off} sec^{-1}	$\frac{K_{off}}{K_{on}} = K'_d$*	K_d(M)†	$\frac{K'_d}{K_d}$	EPMR‡	K (M)§
DMAEB	4.9×10^4	6.5×10^{-4}	1.3×10^{-8}	$(1.3 \pm 0.5) \times 10^{-8}$	1.0	37	$(2.7 \pm 1.7) \times 10^{-8}$
DEAEB	4.1×10^4	3.8×10^{-4}	9.3×10^{-9}	$(6.4 \pm 0.5) \times 10^{-9}$	1.4	18	$(1.2 \pm 0.5) \times 10^{-8}$
4NMPB	2.1×10^5	3.4×10^{-5}	1.6×10^{-10}	$(1.3 \pm 0.8) \times 10^{-10}$	1.2	0.4	$(5.9 \pm 1.0) \times 10^{-10}$
QNB							$(5.1 \pm 0.7) \times 10^{-10}$
Atropine	6.6×10^5	3.3×10^{-4}	5.0×10^{-10}	$(4.9 \pm 2.1) \times 10^{-10}$	1.0	1.4	7.7×10^{-10}
Scopolamine	2.8×10^5	1.2×10^{-4}	4.3×10^{-10}	$(3.5 \pm 0.9) \times 10^{-10}$	1.2	1.0	5.0×10^{-10}

* Determined by kinetic experiments using the guinea pig ileum.
† Determined by equilibrium experiments using the guinea pig ileum. Data are expressed as mean ± S. E. M.
‡ Equipotent molar ratio relative to that of scopolamine in the guinea pig ileum.
§ Dissociation constants calculated from the binding experiments using mouse brain homogenate. Data are expressed as the mean ± S. E. M.

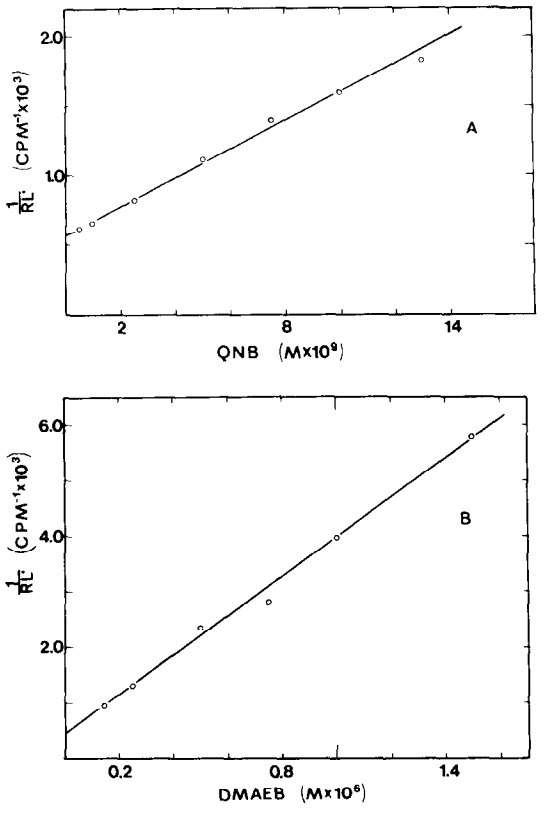


Fig. 7. Competition experiments using [^{3}H]4NMPB and aminoesters of benzilic acid. (A) Competition of 1.7×10^{-9} M [^{3}H]4NMPB with 3-quinuclidinyl benzilate. (B) Competition of 1.7×10^{-9} M [^{3}H]4NMPB with dimethylaminoethyl benzilate.

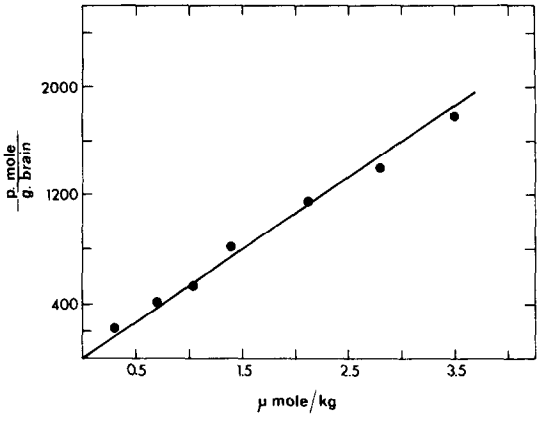


Fig. 8. Uptake of [^{3}H]4NMPB by mouse brain as a function of the dose administered. Mice were injected s.c. with various doses of [^{3}H]4NMPB (6 Ci/m-mole). Twenty min later the mice were decapitated and their brains quickly removed. The concentration of the labelled drug in p moles/g brain was determined as described in Methods and expressed as the mean of two separate experiments (six animals for each point).

others. However, in the two *in vivo* tests scopolamine was found to be ten times more potent than atropine (Tables 2, 3) which is in accord with results reported by Inch [6], Albanus [19] and others. Since an explanation for this apparent discrepancy has been offered in terms of the physiochemical properties of these compounds it was interesting to examine their pharmacokinetic parameters [20]. To this end the time-profile of [^{3}H]scopolamine and [^{3}H]atropine uptake by the mouse brain *in vivo* was investigated. It was found that up to 1.1 per cent of the [^{3}H]scopolamine administered was accumulated in the brain tissue, while only 0.2 per cent of the injected [^{3}H]atropine was taken up. The peak amount of [^{3}H]4NMPB taken up was 1.1 per cent. These values were found to be independent of the dose injected. For example, at doses ranging from 0.35 to 3.5 μmoles/kg, a constant fraction of the administered [^{3}H]4NMPB was taken up, so that the peak amount accumulated in the brain was found to be linearly dependent on the dose injected (Fig. 8).

The decrease in K_d values in the muscarinic preparations caused by incorporation of bulky alkyl groups at the "cationic head" region of the molecules, was found here to coincide with a parallel increase in the "on" and decrease in the "off" rate constants for the antimuscarinic response (Table 4, Fig. 6). It was of interest to see whether this phenomenon is confirmed by published data on large series of antimuscarinic drugs. From the half-life times values reported for the decline of the mydriasis caused in mice by systemic administration, "off" rate constants were calculated assuming a first order kinetics [6]. In addition, using the K_d values of these drugs reported by Inch *et al.* [6] in the guinea pig ileum and using the calculated k_{off} values, the "on" rate constants were also calculated. A log–log plot of the rate and the dissociation constants thus obtained are given in Fig. 9 for the thirty-three drugs tested by Inch *et al.* [6].

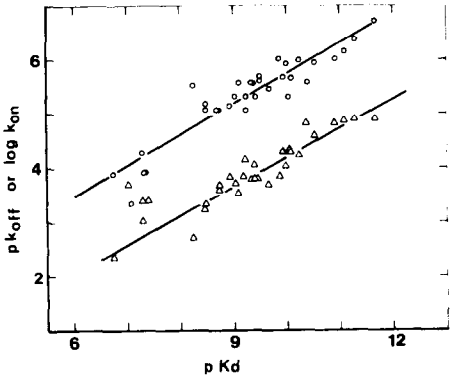


Fig. 9. Treatment of Inch's data as represented in [6]. From the half-life time values determined for the decline of the mydriasis caused in mice by systemic administration, "off" rate constants were computed assuming first order kinetics. Using K_d values of these drugs determined by Inch [6] in the guinea pig ileum and having the K_{off} values, "on" rate constants were calculated. Log K_{on} (○) and $-\log K_{off}$ (pK_{off}) (△) are depicted vs $-\log K_d$ (pK_d).

DISCUSSION

The three pharmacological methods used here for the characterization of the antimuscarinic nature of the aminoesters of benzilic acid are based on

antagonism to a cholinergic agonist, i.e. antagonism to oxotremorine induced hypothermia and disturbances of forced motor activity *in vivo*, and antagonism to the contractile response to acetylcholine of guinea pig ileum *in vitro*. The fourth method is a direct approach in which the affinity of the drugs towards the muscarinic binding-sites from mouse brain homogenate are determined *in vitro*. As shown in Table 5 the concentrations of the drugs taken up by the mouse brain highly exceed their dissociation constants from the muscarinic binding-sites. Therefore, a considerable occupancy of the central muscarinic receptors may be achieved by systemic administration, justifying the comparison of binding constants found *in vitro* with pharmacological values determined *in vivo*.

Structural changes in the aminoalcohol portion of drugs exerting muscarinic acivity are known to markedly influence the activity of both agonists and antagonists [21]. This selectivity of the muscarinic nervous system was studied with two kinds of structural modifications (1) the substitution of dimethyl groups by diethyl groups in dimethylaminoethyl benzilate, (2) the replacement of the aliphatic portion by heterocyclic amino alcohols such as *N*-methyl-4-piperidinol and 3-quinuclidinol. All these compounds were reported previously to exert antimuscarinic activity [5]. The K_d and the ED_{50} values determined for these drugs (Table 4) span over two orders of magnitude—the lower aliphatic analog among the esters of benzilic acid tested, DMAEB, is the least active while the heterocyclic analogs QNB and 4NMPB are the most potent antimuscarinic drugs. These results are in good agreement with those reported by Abood [2], Barlow [22], Inch [6] and recently by Baumgold *et al.* [11] who studied a group of psychotomimetic glycolates using competition experiments with [^{3}H]QNB.

The significant differences in the kinetics of antimuscarinic action *in vivo* and *in vitro* exhibited by the drugs studied here, emphasize the importance of the equilibrium conditions for meaningful comparative studies [6]. The dissociation constants of these drugs in the guinea pig ileum and in mouse brain homogenate (Table 4) were therefore compared here with *in vivo* data obtained at the specific time of the peak effect for each drug (Tables 2, 3). The high degree of similarity found here among the values of dissociation constants of the aminoesters in peripheral and central muscarinic preparations, together with the fairly good correlation established between the *in vivo* and the *in vitro* antimuscarinic potency clearly indicates that the *in vivo* central effects emerged from muscarinic interactions. Moreover, the pattern of antimuscarinic potency was kept constant for the drugs in all four experimental approaches selected here. This finding supports earlier suggestions made by Beld *et al.* [12], Inch *et al.* [6] and Snyder *et al.* [9] that peripheral and central muscarinic receptors are functionally very similar.

The two reference drugs selected here, scopolamine and atropine, seem to represent a special case. Although their chemical structure as well as their spatial architecture are very similar, the central potency of scopolamine is greater than that of atropine by a factor of 10 [23] (Tables 2, 3). This observation, which was traced by the antioxotremorine activity, was reported previously by Mennear *et al.* [24] and by Albanus [1] and discussed recently [20]. Our findings indicate that the fraction of scopolamine and 4NMPB taken up by mouse brain (Table 5) is around 1 per cent of the dose administered while the maximal amount of atropine accumulated does not exceed 0.2% (Table 5). These findings confirm earlier suggestions that in order to raise the amount of atropine to the level believed to be needed to block the central muscarinic receptors, higher doses, relative to scopolamine, have to be systemically administered [20]. Indeed, only at a pharmacological dose of 0.7 mg/kg atropine, is the concentration of this drug in mouse brain comparable to that of scopolamine (Table 5). This seems to validate the pharmacokinetic explanation for the difference between the central activities of atropine and scopolamine, which is based on their physiochemical properties [20].

The data presented in Figs. 6 and 9 is in accordance with several observations regarding the long duration of antimuscarinic activity of these aminoesters in both *in vivo* and *in vitro* preparations. Interestingly, the more potent the drug, the lower is its "off" rate constant; this, in turn, prolongs the duration of the antimuscarinic activity. Indeed, [^{3}H]3-quinuclidinyl-benzilate was found by Snyder *et al.* [25] to be very slowly eliminated from the rat brain. Likewise, Albanus reported on the long duration of anticholinergic symptoms induced in dogs by this and similar drugs [19].

The slow dissociation rate reported here for centrally acting antimuscarinic drugs, may provide the basis for the explanation of a well-known pheno-

Table 5. "Body" and "brain" concentrations of some antimuscarinic drugs

Drug	Body conc. mg/kg*	Body conc. M/kg†	Brain conc. M/kg‡	K_d§ (M)	Brain conc. / K_d
[^{3}H]4NMPB	0.1	2.8×10^{-7}	2.2×10^{-7}	5.9×10^{-10}	370
[^{3}H]Atropine	0.7	1×10^{-6}	1.0×10^{-7}	7.7×10^{-10}	130
[^{3}H]Scopolamine	0.04	1.2×10^{-7}	0.6×10^{-7}	5.0×10^{-10}	120

* Dose of labelled drug used in the uptake experiments.
† Concentration of the labelled drug in the body assuming homogenous distribution.
‡ Concentration of the drug in the brain calculated from the amount of the labelled drug found in the whole brain.
§ From binding experiments.

menon which is not yet fully understood. The transient antidotal activity of physostigmine in man and animals recovering from anticholinergic poisoning by a variety of drugs, including antidepressants [26, 19, 27]. This short-term activity may emerge from the transitional accumulation of endogenous acetylcholine in the CNS which antagonises pharmacologically the antimuscarinic effects. This antagonism is most probably caused by activation of the residual central muscarinic receptors not occupied by the antimuscarinic drug. However, due to the rapid reactivation of the carbamylated cholinesterases and because of the slow dissociation rate of the antimuscarinic drug from the receptors, the antimuscarinic systemic effects are rapidly rebuilt, and repeated physostigmine administration is required.

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REFERENCES

1. L. Albanus, *Acta pharmac. tox.* **28**, 305 (1970).
2. L. G. Abood and J. H. Biel, *Int. Rev. Neurobiol.* **4**, 217 (1962).
3. L. G. Abood, in *Drugs Affecting the Central Nervous System* (Ed. A. Burger), Vol. 2, p. 127, Dekker, NY (1968).
4. R. W Brimblecombe and D. M. Green, *Int. J. Neuropharmac.* **7**, 15 (1968).
5. T. D.Inch and R. W. Brimblecombe, *Int. Rev. Neurobiol.* **16**, 67 (1974).
6. T. D. Inch, D. M. Green and P. B. J. Thompson, *J. Pharm. Pharmac.* **25**, 359 (1973).
7. A. S. V. Burgen and C. R. Hiley, *Br. J. Pharmac.* **51**, 279 (1974).
8. H. I. Yamamura and S. H. Snyder, *Proc. Natn. Acad. Sci. U.S.A.* **71**, 1725 (1974).
9. H. I. Yamamura and S. H. Snyder, *Molec. Pharmac.* **10**, 861 (1974).
10. A. C. Sayers and H. R. Burki, *J. Pharm. Pharmac.* **28**, 252 (1976).
11. J. Baumgold, L. G. Abood and R. Aronstam, *Brain Res.* **124**, 331 (1977).
12. A. J. Beld, S. Van Den Hoven, A. C. Wonterse and M. A. P. Zegers, *Eur. J. Pharmac*, **30**, 360 (1975).
13. A. J. Beld and E. J. Ariens, *Eur. J. Pharmac.* **25**, 203 (1974).
14. J. H. Biel, L. G. Abood, W. K. Hoya, H. H. Leiser, P. A. Nuhfer and E. E. Kluchesky, *J. org. Chem.* **26**, 4096 (1961).
15. Y. Kloog ansd M. Sokolovsky, *Brain Res.* (in press).
16. Edinburgh Staff, in *Pharmacological Experiments on Isolated Preparations*. E. and S. Livingstone, Edinburgh (1970).
17. W. D. M. Paton, *Proc. R. Soc.* **154B**, 21 (1961).
18. F. B. Abramson, R. B. Barlow, F. M. Franks and J. D. M. Pearson, *Br. J. Pharmac.* **51**, 81 (1974).
19. L. Albanus, *FOA Reports* **4**, (1970).
20. H. Weinstein, S. Srebrenik, S. Maayani and M. Sokolovsky, *J. Theor. Biol.* **64**, 295 (1977).
21. M. J. Michelson and E. V. Zeimal, in *Acetylcholine*, p. 73, Pergamon Press, Oxford (1973).
22. R. B. Barlow, F. M. Franks and J. D. M. Pearson, *J. Med. Chem.* **16**, 439 (1973).
23. A. Meyerhöffer, *FOA Reports* **6**, (1972).
24. J. H. Mennear, G. K. Samuel, M. H. Joffe and J. K. Kodama, *Psychopharmacologia* **9**, 347 (1966).
25. H. I. Yamamura, M. J. Kuhar and S. H. Snyder, *Brain Res.* **80**, 170 (1974).
26. J. S. Ketchum, R. F. Sidell, E. B. Crowell, G. K. Aghajanian and A. H. Hayes, *Psychopharmacologia* **28**, 212 (1973).
27. J. S. Burks, J. E. Walker, B. H. Rumack and J. E. Ott, *J. Am. med. Ass.* **230**, 1405 (1974).