MOLECULAR PHARMACOLOGY, 21:594–599

Muscarinic Activity and Receptor Binding of the Enantiomers of Aceclidine and Its Methiodide

BJÖRN RINGDAHL, FREDERICK J. EHLER, AND DONALD J. JENDEN

Department of Pharmacology, University of California School of Medicine, Los Angeles, California 90024

Received October 5, 1981; Accepted January 19, 1982

SUMMARY

The agents (+)- and (−)-aceclidine (3-acetoxyquinuclidine), (+)- and (−)-*N*-methylaceclidine, and oxotremorine were compared in their ability to inhibit specific ^{3}H-labeled (±)-3-quinuclidinyl benzilate binding to rat brain muscarinic receptors and to stimulate contractions of the isolated guinea pig ileum. A good correlation was observed between the high-affinity dissociation constant (K_H) for binding and the muscarinic potency in the isolated ileum. The binding data for the enantiomers of aceclidine were also consistent with their central and peripheral pharmacological activity *in vivo*. Thus the enantiomeric potency ratios [ED_{50} of (−)-aceclidine/ED_{50} of (+)-aceclidine] for the tremorogenic, analgesic, and sialogogic effects in mice agreed well with the ratio K_H of (−)-aceclidine/K_H of (+)-aceclidine.

INTRODUCTION

Recent developments in the pharmacological and biochemical identification of muscarinic receptors in brain tissue have been due mainly to binding studies involving the use of radioactive ligands such as [^{3}H]QNB[1] (1–3). However, it is not possible from such studies alone to determine whether the binding site is equivalent to the physiologically and pharmacologically relevant site. Suggestions of equivalence have come mainly from studies correlating central binding properties with peripheral pharmacological responses (2, 4–7). Direct correlation of binding potency with central functions is difficult, especially for muscarinic agonists, since most potent agonists are quaternary ammonium compounds. However, a correlation between binding affinity and behavioral disturbances was found in a homologous series of antimuscarinic glycolate esters (7).

Aceclidine (3-acetoxyquinuclidine) belongs to the small class of muscarinic agents that are more potent as tertiary amines than as *N*-methyl quaternary ammonium salts (8, 9). Its pharmacological spectrum is similar to those of oxotremorine and arecoline, although the time course of action of the compounds differs (10, 11). Thus, in addition to peripheral effects, e.g., salivation and lacrimation, aceclidine also causes tremor, hypothermia, and analgesia when administered systemically (12). All of these effects are prevented by atropine, whereas quaternary anticholinergic agents are inactive against the tremor and analgesia and only marginally active against the hypothermia (12). These observations suggest a central origin of the tremor and analgesia, and presumably also the hypothermia, induced by aceclidine.

Aceclidine is the only known potent muscarinic agent that is a chiral tertiary amine. It therefore offers the possibility of comparing stereoselectivity obtained from binding experiments with stereoselectivity observed centrally and peripherally *in vivo*. In this study (+)-aceclidine, (−)-aceclidine, and oxotremorine were compared in their ability to inhibit the binding of [^{3}H]QNB to muscarinic receptors in rat brain; their ability to induce tremor, analgesia, hypothermia, and salivation in mice; and their ability to cause contraction of the isolated guinea pig ileum. The binding properties and parasympathomimetic activities of the enantiomers of *N*-methylaceclidine were also compared.

MATERIALS AND METHODS

Drugs. The enantiomers of aceclidine hydrochloride and *N*-methylaceclidine iodide (13) and oxotremorine (14), crystallized as the oxalate salt, were obtained as previously described. Other drugs and their sources were the following: hexamethonium chloride (K&K Laboratories, Plainview, N. Y.), tetram [(2-diethylamino)-ethylphosphorothioic acid *O,O*-diethylester, gift of Dr. R. O'Brien, University of Rochester], and morphine (Merck Laboratories, Rahway, N. J.). Drugs were dissolved in 0.9% NaCl for *in vivo* experiments, in Tyrode's solution for experiments on the ileum, and in distilled water for the binding studies. The Tyrode's solution had the following composition (millimolar): NaCl, 137; $NaHCO_3$, 12; glucose, 5.0; KCl, 2.7; $MgSO_4 \cdot 7\ H_2O$, 1.0; NaH_2PO_4, 0.4; $CaCl_2$, 1.8.

Muscarinic activity in intact mice. The median effective dose of the test compounds to produce tremor and

This work was supported by United States Public Health Service Grant MH-17691.

[1] The abbreviation used is: QNB, (±)-3-quinuclidinyl benzilate.

0026-895X/82/030594-06$02.00/0

salivation was estimated by the "up-and-down" method for small samples described by Dixon (15). A logarithmic series of doses, with a spacing of 0.1 unit in the $\log_{10}$ scale, was given by i.v. injection to groups of Swiss-Webster mice weighing 27–32 g. The presence or absence of tremors and salivation was determined by visual inspection during the first 5 min after drug administration.

Analgesic effect in mice. Hot-plate reaction times were measured by a method similar to that of Woolfe and MacDonald (16). The plate was maintained at 58 ± 1°. Lifting of the hind limb was taken as a positive response to the noxious stimulus. A maximal cutoff time of 30 sec was used. Drugs were administered i.v. to groups of six mice at three dose levels. The reaction time was determined at 5-min intervals until it had returned to that of the control group. Dose-response curves were constructed from the mean of the integrated response time between 0 and 15 min. The potency was expressed as the dose, calculated by linear regression analysis, required to double the integrated response time relative to the control.

Hypothermic effect in mice. The test compounds were administered i.v. to groups of five mice at five dose levels. One group was treated with 0.9% NaCl. Rectal temperature was recorded with a Tele-thermometer (Yellow Springs Instrument Company, Yellow Springs, Ohio) at constant ambient temperature (23.5 ± 0.5°). The rectal temperature of each animal was read 30 sec after insertion of the probe approximately 30 mm from the anus. The hypothermic effect was expressed as the mean of the decrease in temperature relative to preinjection temperature of each group.

Acute toxicity in mice. LD_{50} values were determined by i.v. injection using the "up-and-down" method for small samples (15). Mortality counts were taken after 15 min.

Isolated guinea pig ileum. Male guinea pigs (English short hair, 350–450 g) were killed by a blow to the head. Segments of ileum (2–3 cm long) were removed and suspended in a 10-ml organ bath containing Tyrode's solution at 37° and aerated with O_2 containing 5% CO_2. Contractions were recorded isotonically at 1 g of tension, using an electromechanical displacement transducer and a potentiometric recorder.

The enantiomers of aceclidine and *N*-methylaceclidine were compared with oxotremorine as a reference compound on pieces of ileum taken from different guinea pigs using the cumulative dose-response technique. The same experiment was repeated after exposure of the preparation to hexamethonium (0.3 mM). In separate experiments, the tissue was incubated with tetram (50 μM) for 1 hr and then washed for 1 hr with Tyrode's solution containing morphine (3 mM), which was used thereafter for all washes. Dose-response curves to the various agonists were then obtained as described above.

Binding characterization. Muscarinic receptor binding assays were performed on rat brain stem homogenates. The brain stem was homogenized in 50 mM sodium-potassium phosphate buffer (81 mM Na^+, 9.5 mM K^+, 50 mM PO_4, pH 7.4) with a glass homogenizer (Potter Elvejhem) and Teflon pestle. The final homogenate concentration was 40 mg/ml (original wet tissue weight). The binding of [^{3}H]QNB (29 Ci/mmole; New England Nuclear Corporation, Boston, Mass.) was measured according to the procedure of Yamamura and Snyder (1), with minor modifications. Routinely, 100 μl of brain stem homogenate were incubated with [^{3}H]QNB in a final volume of 2 ml containing 50 mM sodium-potassium phosphate buffer (pH 7.4). Incubations lasted 60 min at 37°. Binding in the presence of 10 μM atropine was defined as nonspecific. For measurement of the competitive inhibition of [^{3}H]QNB binding by nonlabeled ligands, a total concentration of 0.8 or 1.6 nM [^{3}H]QNB was used. At these concentrations less than 5% of the total [^{3}H]QNB was bound.

The binding parameters were determined from the experimental data by nonlinear least-squares regression analysis using the computer program NONLIN (17). The ligand/[^{3}H]QNB competitive inhibition data were fitted to the following two-site competitive inhibition equation (3):

$$B = \frac{a}{1 + x/K'_H} + \frac{1 - a}{1 + x/K'_L} \quad (1)$$

in which B is the proportion of [^{3}H]QNB bound, a is the proportion of high-affinity sites, K'_H and K'_L are the apparent dissociation constants of the high- and low-affinity sites, and x is the concentration of nonlabeled inhibitor. In some instances the competitive inhibition data were adequately described by the following one-site competitive inhibition equation:

$$B = \frac{1}{1 + x/K'} \quad (2)$$

where K' is the apparent dissociation constant of the nonlabeled ligand. The apparent dissociation constants (K') were corrected to give the true dissociation constants (K) by the following relationship:

$$K = \frac{K'}{1 + y/K_{QNB}} \quad (3)$$

In Eq. 3, y is the concentration of [^{3}H]QNB, and K_{QNB} is the dissociation constant of [^{3}H]QNB which was assigned a value of 1.4×10^{-10} M. This value was determined independently by Scatchard analysis of five-point [^{3}H]QNB binding isotherms. The IC_{50} values of nonlabeled ligands (concentration of nonlabeled ligand that caused half-maximal inhibition of specific [^{3}H]QNB binding) were determined by analysis of the ligand/[^{3}H]QNB competition curves. These IC_{50} values were corrected for receptor occupancy by [^{3}H]QNB according to the following relationship:

$$K_i = \frac{IC_{50}}{1 + y/K_{QNB}} \quad (4)$$

in which K_i is the concentration of nonlabeled ligand that causes half-maximal receptor occupation in the absence of [^{3}H]QNB. For those cases in which the ligand/[^{3}H]QNB competition data were adequately described by the one-site competition equation (Eq. 2), IC_{50} and K_i are essentially equivalent to K' and K, respectively.

RESULTS

As shown in Table 1, (+)-aceclidine was more potent than (−)-aceclidine in producing tremors and salivation in mice. In both of these effects, (+)-aceclidine was less potent than oxotremorine. The effects appeared almost immediately after injection. The duration of the tremor (less than 5 min) and salivation (5–15 min) induced by (+)-aceclidine was short compared with the duration of these effects after administration of oxotremorine. With (+)-aceclidine the ED_{50} value for tremor was significantly higher than that for salivation. In contrast, for oxotremorine there was no significant difference between the tremorogenic and sialogogic doses.

Oxotremorine produced analgesia at doses below those at which tremor and salivation were observed (Table 1). The analgesia induced by (+)- and (−)-aceclidine was of shorter duration and only appeared at doses that caused profound salivation.

(+)-Aceclidine was more potent than (−)-aceclidine in producing hypothermia in mice (Table 1). Figure 1 illustrates the hypothermic effects of (+)-aceclidine and oxotremorine. Injection of 2 μmoles/kg of (+)-aceclidine produced maximal hypothermia at 5–15 min. With 8 μmoles/kg, the maximal hypothermia occurred at 15–25 min. Higher doses did not cause a further decrease in body temperature. The hypothermic effect of oxotremorine was more profound and longer lasting. (−)-Aceclidine induced only a minor, short-lasting hypothermia, with maximal response occurring at doses near the LD_{50} (Table 1).

On the isolated guinea pig ileum, (+)- and (−)-aceclidine were more potent than (+)- and (−)-*N*-methylaceclidine, but less potent than oxotremorine (Table 2). Hexamethonium (0.3 mM) had very little effect on the potencies of the compounds. (+)-Aceclidine was about 14 times as active as (−)-aceclidine in the untreated ileum. After treatment of the ileum with tetram (an irreversible anticholinesterase agent) and morphine, the enantiomeric potency ratio was reduced by a factor of 2 because of a greater relative potency of the (−)-isomer. (+)-*N*-Methylaceclidine was not capable of producing a maximal contraction in either tissue. The dose-response curve obtained with (−)-*N*-methylaceclidine was found to be parallel to those of (+)- and (−)-aceclidine and oxotremorine and to attain the same maximal response value. The relative potency of (−)-*N*-methylaceclidine was increased almost 10-fold in the anticholinesterase-treated tissue (Table 2).

The competitive inhibition of [^{3}H]QNB binding by oxotremorine and the enantiomers of aceclidine and *N*-methylaceclidine is illustrated in Fig. 2. As shown in Fig. 2, the enantiomers of aceclidine were less potent than oxotremorine but more potent than the enantiomers of *N*-methylaceclidine. (+)-Aceclidine was the more potent of the aceclidine isomers. The K_i values of oxotremorine, (+)-aceclidine, (−)-aceclidine, (+)-*N*-methylaceclidine, and (−)-*N*-methylaceclidine were 0.24, 3.5, 12, 49, and 43 μM, respectively. The competition curves of oxotremorine and (+)-aceclidine were flatter than expected for simple mass-action behavior and were adequately described by a two-site binding equation. In contrast, the competition curves of the other enantiomers were consistent with a one-site binding equation. The binding parameters of the compounds were determined from the competition curves by nonlinear regression, and the results of this analysis are shown in Table 3.

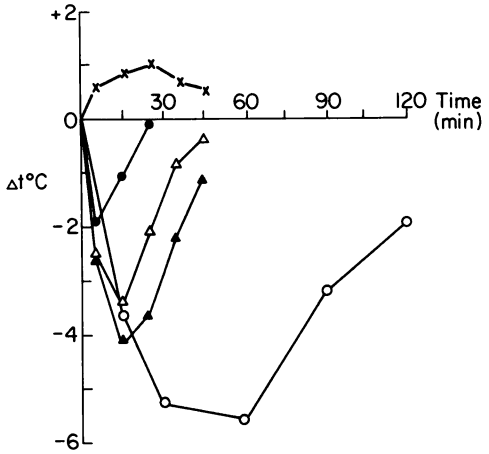

FIG. 1. *Hypothermic effect of (+)-aceclidine and oxotremorine in mice (i.v.)*

(+)-Aceclidine: 2.0 (●), 4.0 (△), and 8.0 (▲) μmoles/kg; oxotremorine: 1.0 μmol/kg (○); control: 0.9% NaCl (×).

DISCUSSION

Several authors have investigated the enantiomers of aceclidine and *N*-methylaceclidine for muscarine-like activity on the isolated guinea pig ileum (19–21). Although there is general agreement that (*S*)-(+)-aceclidine is more potent than (*R*)-(−)-aceclidine in this tissue, the results obtained with the enantiomers of *N*-methylaceclidine are somewhat contradictory. Barlow and Casy (19) and Lambrecht (21) reported, and their results are confirmed in the present study, that the (*R*)-(−)-isomer

TABLE 1

Acute toxicity and tremorogenic, sialogogic, analgesic, and hypothermic activity of (+)-aceclidine, (−)-aceclidine, and oxotremorine in mice

Compounds were administered i.v. Each value represents the mean ± standard error of the mean.

Compound	Acute toxicity LD_{50}	Tremors ED_{50}	Salivation ED_{50}	Analgesia[a]	Hypothermia[b]
			μmoles/kg		
(+)-Aceclidine	32 ± 3	5.9 ± 1.2	1.0 ± 0.18	3.3 ± 0.8	8.0 (−4.2 ± 0.5)
(−)-Aceclidine	345 ± 52	246 ± 37	35.4 ± 4.6	201 ± 100	315 (−1.4 ± 0.4)
Oxotremorine	6.8[c]	0.27 ± 0.05	0.19 ± 0.07	0.057 ± 0.022	2.0 (−7.3 ± 1.0)

[a] Dose required to double the integrated hot-plate response time relative to the control (see Materials and Methods).

[b] Lowest dose giving maximal hypothermic response (Δ *t*°).

[c] Reference 18.

TABLE 2

Muscarinic activity of oxotremorine and the enantiomers of aceclidine and N-methylaceclidine in the isolated guinea pig ileum

Each value represents the mean ± standard deviation of at least four estimates.

Compound	ED_{50}	EPMR[a]	EPMR[a, b]
	moles/liter		
(+)-Aceclidine	$(2.4 \pm 0.3) \times 10^{-7}$	5.0 ± 0.6	5.7 ± 1.1
(−)-Aceclidine	$(3.4 \pm 0.4) \times 10^{-6}$	71 ± 8	34.5 ± 3.7
(+)-*N*-Methylaceclidine	$>5 \times 10^{-4}$	$>10^4$	$>10^4$
(−)-*N*-Methylaceclidine	$(5.9 \pm 1.5) \times 10^{-5}$	1280 ± 310	146 ± 10
Oxotremorine	$(4.4 \pm 0.9) \times 10^{-8}$	1.0	1.0

[a] Equipotent molar ratio.

[b] In anticholinesterase-treated tissue, as described under Materials and Methods.

of *N*-methylaceclidine is more potent than the (*S*)-(+)-isomer. Thus *N*-methylation of the tertiary base caused an inversion of the stereoselectivity for the receptor. On the other hand, Weinstein *et al.* (20) found that (*S*)-(+)-*N*-methylaceclidine is somewhat more active than the (*R*)-(−)-isomer, implying that no such inversion of stereoselectivity occurs. (*R*)-(−)-Aceclidine (22) and (*R*)-(−)-*N*-methylaceclidine (23) are rather good substrates for acetylcholinesterase, but none of the above results were obtained from anticholinesterase-treated tissues.

Some of our estimates of the muscarinic activities of the enantiomers of aceclidine and *N*-methylaceclidine were made in the presence of morphine, which inhibits acetylcholine release from nerve endings (24), and after treatment with an anticholinesterase agent. Under these conditions the order of potency of the compounds was the same as in the untreated tissue. However, the relative potencies of the enantiomers reported (22, 23) to be substrates for acetylcholinesterase were increased, leading to a lower enantiomeric potency ratio for the aceclidine isomers and a higher potency ratio for the *N*-methylaceclidine isomers. The results confirm that an inversion of stereoselectivity for the receptor takes place on *N*-methylation of (+)- and (−)-aceclidine (19, 21).

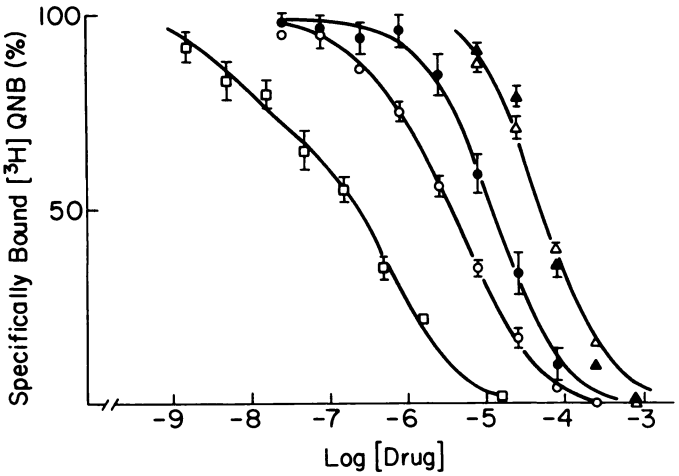

FIG. 2. *Competitive inhibition of [^{3}H]QNB binding by oxotremorine (□), (+)-aceclidine (○), (−)-aceclidine (●), (+)-N-methylaceclidine (△), and (−)-N-methylaceclidine (▲)*

Mean binding values ± standard error of the mean of at least three experiments are shown. The competition curves have been corrected for the shift caused by receptor occupancy by [^{3}H]QNB. The oxotremorine/[^{3}H]QNB competition experiments were carried out at a [^{3}H]QNB concentration of 0.8 nM, whereas the other experiments were performed at a [^{3}H]QNB concentration of 1.6 nM. The theoretical curves represent the least-squares fit to the data. The theoretical curves for the enantiomers of *N*-methylaceclidine were nearly superimposable; hence, only one curve is shown for these two enantiomers.

The pharmacological potency, as measured by the contractile responses in the guinea pig ileum, is determined by both affinity and efficacy. The efficacy of (−)-*N*-methylaceclidine is greater than that of (+)-*N*-methylaceclidine, since the latter behaved as a partial agonist. The difference in pharmacological potency between the two compounds thus appears to be due mainly to a difference in efficacy. It is not surprising, therefore, that the binding affinities of the enantiomers of *N*-methylaceclidine were similar.

In a study investigating the binding of agonists to rat brain muscarinic receptors, a strong correlation between the K_H and pharmacological activity was observed (25). Therefore, we compared the K_H of oxotremorine and the enantiomers of aceclidine and *N*-methylaceclidine with their pharmacological activity in the isolated guinea pig ileum. The results of this comparison are shown graphically in Fig. 3. The tendency for the points to fall on the line of equivalence ($Y = X$) demonstrates a strong correlation ($r = 0.92$; $p = 0.03$) between the binding parameters of these compounds and their pharmacological activities.

(+)-Aceclidine binding, like the binding of oxotremorine, was consistent with a two-site binding equation, whereas the binding of (−)-aceclidine and (+)- and (−)-*N*-methylaceclidine fit a one-site equation. These data suggest that the efficacy of (+)-aceclidine is greater than that of the other enantiomers, since a correlation between K_L/K_H and efficacy has been noted (25).

The binding data for the enantiomers of aceclidine were also consistent with their pharmacological activities *in vivo*. Thus the enantiomer which has the greater pharmacological activity (tremor, salivation, analgesia, and hypothermia) also had greater potency in the [^{3}H]QNB binding assay.

The hypothermia and analgesia induced by (±)-aceclidine were previously shown to be prevented by atropine, but only marginally reduced by methylatropine (12). These observations suggest a central muscarinic involvement in the hypothermic and analgesic effects. Further support for a central muscarinic component in these effects is provided by the similar stereoselectivity shown by the enantiomers of aceclidine in the hypothermic, analgesic, and tremorogenic effects, the latter apparently of central muscarinic origin (11). Although the enantiomers of aceclidine differed widely in their hypothermic effects, no exact enantiomeric potency ratio could be given since they do not produce the same maximal effect (Table 1). The enantiomeric potency ratios for the tremorogenic (ratio 45), analgesic (ratio 61), and sialogogic

TABLE 3

Binding parameters of oxotremorine and the enantiomers of aceclidine and N-methylaceclidine

The binding parameters were determined by nonlinear regression analysis of three competitive binding experiments.

Compound	High-affinity sites	K_H	K_L	K_L/K_H
	%	moles/liter		
(+)-Aceclidine	26	3.4×10^{-7}	6.6×10^{-6}	20
(−)-Aceclidine		1.2×10^{-5}		
(+)-*N*-Methylaceclidine		4.9×10^{-5}		
(−)-*N*-Methylaceclidine		4.3×10^{-5}		
Oxotremorine	30	5.1×10^{-9}	5.5×10^{-7}	107

(ratio 35) effects agree remarkably well with the ratio K_H (−)-aceclidine/K_H (+)-aceclidine (ratio 35). These findings provide good evidence of the structural integrity of the isolated receptors.

The relationship between the ED_{50} for smooth muscle contraction and the binding parameters of muscarinic agonists is not yet fully understood (3, 6, 25). According to Birdsall *et al.* (25), it is unlikely that the high-affinity binding site is the relevant site for smooth muscle contraction because of the existence of spare receptors for muscarinic agonists with respect to contractile responses. When spare receptors are considered, the ED_{50} for smooth muscle contraction generally agrees with K_L (25). Beld *et al.* (26) have criticized this interpretation. They suggested that the high-affinity binding site is the one directly involved in mediating the biological response. Our results do not permit a distinction to be made between the two models. However, it is obvious that the pharmacological potency *in vivo* and *in vitro* is correlated with the high-affinity dissociation constant (K_H).

The threshold doses of oxotremorine for central (tremor) and peripheral (salivation) muscarinic activity are nearly identical (Table 1). Similar results were obtained by Herz *et al.* (27), who also showed that the same is true for arecoline, although the central effects of arecoline are less pronounced (11). With (+)-aceclidine, tremors were seen only at doses much above those which cause salivation (Table 1). The rate of penetration of tertiary amines into the central nervous system is determined *inter alia* by the proportion of the un-ionized form (28, 29). We suggest that the relatively low central specificity of (+)-aceclidine is due mainly to its higher base strength (pK_a 8.93) (9) as compared with that of oxotremorine (pK_a 7.91) (30) and arecoline (pK_a 7.72) (30).

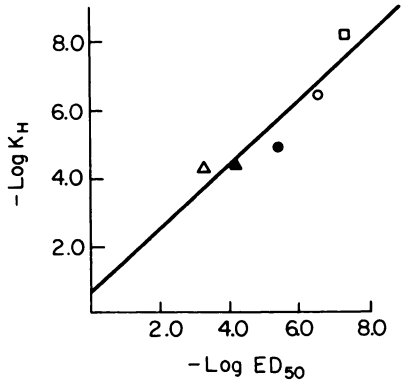

FIG. 3. *Correlation of the high-affinity dissociation constant and pharmacological activity of (+)-aceclidine (○), (−)-aceclidine (●), (+)-N-methylaceclidine (△), (−)-N-methylaceclidine (▲), and oxotremorine (□)*

Ordinate, the negative logarithm of the K_H (Table 3); abscissa, the negative logarithm of the ED_{50} for contraction of the guinea pig ileum (Table 2).

ACKNOWLEDGMENTS

We wish to acknowledge the excellent editorial assistance of Mrs. F. Comes and D. Matushek.

REFERENCES

1. Yamamura, H. I., and S. H. Snyder. Muscarinic cholinergic binding in rat brain. *Proc. Natl. Acad. Sci. U. S. A.* **71**:1725–1729 (1974).
2. Snyder, S. H., K. J. Chang, M. J. Kuhar, and H. I. Yamamura. Biochemical identification of the mammalian muscarinic cholinergic receptor. *Fed. Proc.* **34**:1915–1921 (1975).
3. Birdsall, N. J. M., A. S. V. Burgen, and E. C. Hulme. Muscarinic receptors: biochemical binding studies, in *Recent Advances in Receptor Chemistry* (F. Gualtieri, M. Giannella, and C. Melchiorre, eds.). Elsevier/North-Holland Biomedical Press, Amsterdam, 71–96 (1979).
4. Aronstam, R. S., D. J. Triggle, and M. E. Eldefrawi. Structural and stereochemical requirements for muscarinic receptor binding. *Mol. Pharmacol.* **15**:227–234 (1979).
5. Hulme, E. C., N. J. M. Birdsall, A. S. V. Burgen, and P. Mehta. The binding of antagonists to brain muscarinic receptors. *Mol. Pharmacol.* **14**:737–750 (1978).
6. Birdsall, N. J. M., and E. C. Hulme. Biochemical studies on muscarinic acetylcholine receptors. *J. Neurochem.* **27**:7–16 (1976).
7. Baumgold, J., L. G. Abood, and R. Aronstam. Studies on the relationship of binding affinity to psychoactive and anticholinergic potency of a group of psychotomimetic glycolates. *Brain Res.* **124**:331–340 (1977).
8. Mashkovsky, M. D., and L. N. Yakhontov. Relationships between chemical structure and pharmacological activity in a series of synthetic quinuclidine derivatives. *Prog. Drug Res.* **13**:293–339 (1969).
9. Cho, A. K., D. J. Jenden, and S. I. Lamb. Rates of alkaline hydrolysis and muscarinic activity of some aminoacetates and their quaternary ammonium analogs. *J. Med. Chem.* **15**:391–394 (1972).
10. Jenden, D. J. Studies on tremorine antagonism by structurally related compounds, in *Biochemistry and Pharmacology of the Basal Ganglia* (E. Costa, L. J. Cote, and M. D. Yahr, eds.). Raven Press, New York, 159–170 (1966).
11. Jenden, D. J. Testing of drugs for therapeutic potential in Parkinson's disease, in *Methods of Pharmacological Testing* (A. Burger, ed.), Vol. 3. Marcel Dekker, New York, 337–361 (1968).
12. Chiang, T. S., and F. E. Leaders. Antagonism of aceclidine-induced tremor, analgesia, hypothermia, salivation and lacrimation by some pharmacological agents. *Arch. Int. Pharmacodyn. Ther.* **189**:295–302 (1971).
13. Ringdahl, B., B. Resul, and R. Dahlbom. Facile preparation of the enantiomers of 3-acetoxyquinuclidine and 3-quinuclidinol. *Acta Pharm. Suec.* **16**:281–283 (1979).
14. Bebbington, A., and D. Shakeshaft. An improved synthesis of oxotremorine. *J. Med. Chem.* **8**:274–275 (1965).
15. Dixon, W. J. The up and down method for small samples. *J. Am. Statist. Assoc.* **60**:967–978 (1965).
16. Woolfe, G., and A. D. MacDonald. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). *J. Pharmacol. Exp. Ther.* **80**:300–307 (1944).
17. Metzler, C. M., G. L. Elfring, and A. J. McEwan. A User's Manual for NONLIN. The Upjohn Company, Kalamazoo, Mich. (1974).
18. Bebbington, A., R. W. Brimblecombe, and D. Shakeshaft. The central and peripheral activity of acetylenic amines related to oxotremorine. *Br. J. Pharmacol.* **26**:56–67 (1966).
19. Barlow, R. B., and A. F. Casy. Inversion of stereospecificity by methylation

of compounds acting at acetylcholine receptors. *Mol. Pharmacol.* 11:690–693 (1975).
20. Weinstein, H., S. Maayani, S. Srebrenik, S. Cohen, and M. Sokolovsky. A theoretical and experimental study of the semirigid cholinergic agonist 3-acetoxyquinuclidine. *Mol. Pharmacol.* 11:671–689 (1975).
21. Lambrecht, G. Struktur und Konformations-Wirkungs-Beziehungen Heterocyclischer Acetylcholinanaloga. 1. Muskarinwirkung enantiomerer 3-Acetoxychinuclidine und 3-Acetoxypiperidine. *Eur. J. Med. Chem.* 11:461–466 (1976).
22. Pyttel, R., and J. B. Robinson. Interaction of 3-quinuclidinol and its derivatives with acetylcholinesterase. *J. Pharm. Sci.* 62:684–685 (1973).
23. Robinson, J. B., B. Belleau, and B. Cox. 3-Acetoxyquinuclidine methiodide: resolution, absolute configuration and stereospecificity of interaction with the acetylcholine binding sites. *J. Med. Chem.* 12:848–851 (1969).
24. Paton, W. D. M. The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea pig ileum. *Br. J. Pharmacol.* 12:119–127 (1957).
25. Birdsall, N. J. M., A. S. V. Burgen, and E. C. Hulme. The binding of agonists to brain muscarinic receptors. *Mol. Pharmacol.* 14:723–736 (1978).
26. Beld, A. J., E. J. Klok, and J. F. Rodrigues de Miranda. Inhibition of [^{3}H] dexetimide binding by a homologous series of methylfurthrethonium analogues at the peripheral muscarinic receptor. *Biochem. Biophys. Res. Commun.* 97:430–436 (1980).
27. Herz, A., H. Holzhauser, and T. Teschemacher. Zentrale und periphere Wirkungen von Cholinomimetica und ihre Abhängigkeit von der Lipoidlöslichkeit. *Arch. Exp. Pathol. Pharmakol.* 253:280–297 (1966).
28. Brodie, B. B., H. Kurz, and L. S. Shanker. The importance of dissociation constant and lipid solubility in influencing the passage of drugs into the cerebrospinal fluid. *J. Pharmacol. Exp. Ther.* 130:20–25 (1960).
29. Karlen, B., and D. J. Jenden. The role of distribution as a determinant of central anticholinergic specificity in a series of oxotremorine analogs. *Res. Commun. Chem. Pathol. Pharmacol.* 1:471–478 (1970).
30. Hanin, I., D. J. Jenden, and A. K. Cho. The influence of pH on the muscarinic action of oxotremorine, arecoline, pilocarpine and their quaternary ammonium analogs. *Mol. Pharmacol.* 2:352–359 (1966).

Send reprint requests to: Dr. Donald J. Jenden, Department of Pharmacology, University of California School of Medicine, Los Angeles, Calif. 90024.