Stereochemical Preferences for Curarimimetic Neuromuscular Junction Blockade I: Enantiomeric Monoquaternary Amines as Probes

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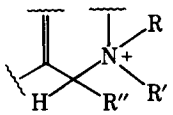
Abstract □ Seven pairs of monoquaternary enantiomeric neuromuscular junction blocking agents were prepared in which the carbon asymmetry is adjacent to the quaternized nitrogen moiety. The tertiary amines from which the blocking species were obtained are carnegine, laudanosine, *N*-methylpavine, corydine, isocorydine, glaucine, and boldine. Curarimimetic potencies, obtained with an *in vivo* cat tongue–hypoglossal nerve preparation, were obtained for the enantiomeric methiodides of each of these amines. Possible contributions to activity by preferential binding to blood components or by selective inhibition of acetylcholinesterase also were studied. The combined studies indicate that there is a modest preference by the neuromuscular junction of the cat for monoquaternary blockers with the *(S)*-configuration.

Keyphrases □ Curarimimetic activity—stereochemical preferences for neuromuscular junction blockade, seven pairs of enantiomeric monoquaternary amines (carnegine, laudanosine, *N*-methylpavine, corydine, isocorydine, glaucine, and boldine as methiodides) as probes □ Neuromuscular junction blockade (curarimimetic)—stereochemical preferences, seven pairs of enantiomeric monoquaternary amines as methiodides as probes □ Amines, enantiomeric monoquaternary—as probes for determination of stereochemical preferences for curarimimetic neuromuscular junction blockade □ Blocking agents, neuromuscular—carnegine, laudanosine, *N*-methylpavine, corydine, isocorydine, glaucine, and boldine (as methiodides), stereochemical preferences for curarimimetic neuromuscular junction blockade, enantiomeric monoquaternary amines as probes

The structural requirements for neuromuscular junction blockade have been a heavily researched area in the study of chemical activity on physiological function (1). Most applicable parameters have received intensive study, and it is surprising that little has been done regarding enantiomeric comparisons (2). The following structure illustrates the nature of the asymmetry commonly possessed by curarimimetic neuromuscular junction blockers:



The possibility that carbon asymmetry adjacent to the quaternary nitrogen may cause potency differences among the stereochemical isomers was indicated by the work of King (3), who found that (+)-tubocurarine was 20–60 times more potent than (−)-tubocurarine. Similarly, earlier work (4) indicated that nitrogen asymmetry, obtained by methyl quaternization of canadine, can also lead to differing neuromuscular junction blocking potencies among the various stereochemical isomers.

Since these initial findings, support for such a premise has been rather sparse since a systematic investigation of the effects of asymmetry on curarimimetic activity is nonexistent. However, in cases where configurational isomerism was encountered, different potencies among the isomers also were observed (2, 5–9).

This series of studies addresses itself to the elucidation of stereochemical parameters, resulting from configurational isomerism, that may affect neuromuscular junction blocking potency. Asymmetry *per se* is not necessary for blocking activity, and these studies are not presupposed on finding such differences. Instead, they are based on establishing the extent, if any, of such differences in the hope of further defining the characteristics of the binding site for these types of blocking agents.

In beginning such a study, it seemed reasonable to start at the simplest level, that is to employ as probes molecules possessing only a single asymmetric atom. In terms of pharmacological design and for synthetic simplicity, it was decided to pattern the probes after a *hemi*-curare type of structure. The resulting tetrahydroisoquinoline types, after quaternization, would then bear an onium head in a "pachy-curare" arrangement typical of curarimimetic agents. This approach was taken by earlier workers (10, 11), who found that such structural types do indeed show weak curare-like activity. The synthesized probes (I–XIV) were subjected to bioassay in this initial work.

Further studies will employ more complex probes and will consider the stereochemical preferences of bisquaternaries with asymmetric carbon atoms, monoquaternaries with carbon–nitrogen asymmetry and simple nitrogen asymmetry, and bisquaternaries with carbon–nitrogen asymmetry and uncomplicated nitrogen asymmetry.

CHEMISTRY

The laudanosinium (I and II) and *N,N*-dimethylpavinium (III and IV) probes were synthesized from papaverine. Treatment of papaverine methiodide with methanolic sodium borohydride (12) produced (±)-laudanosine. Its resolution was achieved *via* the *O,O*-di-*p*-toluoyl bitartrate salts (13). Deracemization with quinic acid (14, 15), tartaric acid, and *O,O*-dibenzoyltartaric acid (16) was unsuccessful. Treatment of papaverine methiodide with sodium borohydride in pyridine (17) yielded the partially reduced 1,2-dihydro compound, which was cyclized to (±)-*N*-methylpavine by the usual acid treatment (18). Resolution was accomplished *via* the bitartrate salts (19). Methyl iodide quaternization of each enantiomeric pair produced the desired probe compounds.

The carneginium probes (V and VI) were synthesized from *N*-acetylhomoveratrylamine which, under Bischler–Napieralski reaction conditions (20) and subsequent reduction with sodium borohydride in methanol (21), yielded (±)-salsolidine. This compound was deracemized *via* the *O,O*-dibenzoyl bitartrate salts (21).

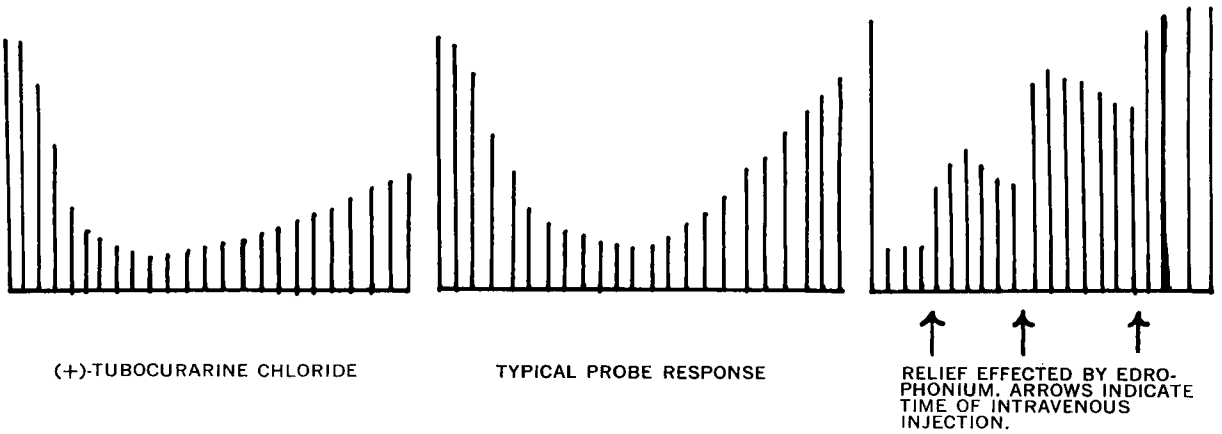
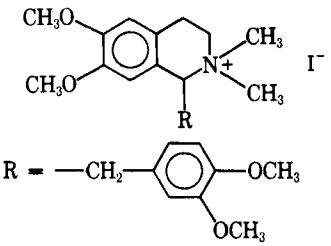


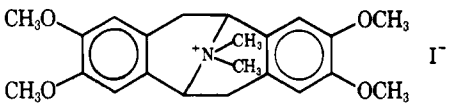
Figure 1—*Pattern of neuromuscular junction block onset, duration, and relief.*

Methyl iodide treatment of each enantiomer under buffered conditions provided the desired probe pair.

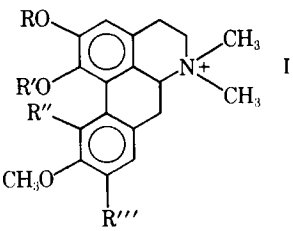
The aporphinium-type probes were prepared from the commercially available, naturally occurring tertiary bases, all of which possessed the *(S)*-configuration. Methyl iodide quaternization of these [(+)-glaucine, (+)-corydine, (+)-isocorydine, and (+)-boldine] gave the *(S)*-component of the desired enantiomeric probe pairs (VII, IX, XI, and XIII). Racemization of the optically active



I: (*S*)-*N*-methyllaudanosinium iodide
II: (*R*)-*N*-methyllaudanosinium iodide
R = CH_3
V: (*S*)-*N*-methylcarneginium iodide
VI: (*R*)-*N*-methylcarneginium iodide



III: (*S,S*)-*N,N*-dimethylpavinium iodide
IV: (*R,R*)-*N,N*-dimethylpavinium iodide



R = R′ = CH_3, R″ = H, R‴ = OCH_3
VII: (*S*)-*N*-methylglaucinium iodide
VIII: (*R*)-*N*-methylglaucinium iodide
R = CH_3, R′ = R‴ = H, R″ = OCH_3
IX: (*S*)-*N*-methylcorydinium iodide
X: (*R*)-*N*-methylcorydinium iodide
R = R′ = CH_3, R″ = OH, R‴ = H
XI: (*S*)-*N*-methylisocorydinium iodide
XII: (*R*)-*N*-methylisocorydinium iodide
R = R″ = H, R′ = CH_3, R‴ = OH
XIII: (*S*)-*N*-methylboldinium iodide
XIV: (*R*)-*N*-methylboldinium iodide

bases was effected by the catalytic hydrogenation (22) in acetic acid (21). Deracemization of the racemates of glaucine, corydine, and isocorydine was accomplished *via* the bitartrate route suggested (23) for glaucine.

Although many attempts were made to resolve the boldine racemate, it was only possible through the *O,O*-di-*p*-toluoyl bitartrate salts. Again, methyl iodide quaternization of the resultant *(R)*-enantiomers was successful and provided the other half of the required enantiomeric probe pairs (VIII, X, XII, and XIV). All of the compounds prepared, other than known compounds, were characterized by melting point, optical rotation, TLC, and spectral methods (IR, NMR, UV, and mass spectroscopy). Final compounds were also subjected to elemental analysis. All analytical and spectral data were in accord with known values. Melting point, optical rotation, and TLC data were obtained again, after physiological testing, to ensure that the quality of the test probes had not changed.

BIOLOGY

In the selection of an assay for neuromuscular junction blocking abilities, two considerations led to the decision that an *in vivo* cat preparation would be the most appropriate:

1. It is well established (24–26) that among various species the cat most closely resembles humans in its response to neuromuscular junction blocking agents.
2. Maclogan (27) pointed out that isolated nerve–muscle preparations do not simulate the intact animal in overall response, duration of action, and recovery patterns.

Although several *in vivo* cat preparations have been described, they all employ a muscle removed from its natural surroundings and necessitate precautionary measures such as the maintenance of a controlled muscle temperature (28–31). However, the hypoglossal nerve–tongue muscle preparation allows the muscle to be maintained in its natural surroundings, and previous experience in these laboratories (21) established its suitability for this type of study[1].

In assessing the nature of the blockade produced by the monoquaternary probes, several observations support the conclusion that the block is of a curarimimetic type:

1. No initial contraction or muscular fasciculation, characteristic of depolarizing-type blockers in their action on cat muscle (1), was observed.
2. The blocking patterns (Fig. 1) of the probes were identical to those of (+)-tubocurarine in their onset of action and decay to maximal effect, although (+)-tubocurarine had a much longer duration of action which manifested itself in a recovery phase with a more gentle slope.
3. The blocks established by the probes and by (+)-tubocurarine were shown to be cumulative at low doses.
4. The blocks established by (+)-tubocurarine and by the

[1] The method has been used as a teaching tool in the pharmacology laboratories of the University of Minnesota and Michigan State University, but it apparently has not been described in the literature. Therefore, a detailed description of this method appears in the *Experimental* section.

Table I—Neuromuscular Junction Blocking Potencies

Drug	r^{2a}	ED_{50}, $\times 10^{-4}$ mmole/kg	Duration, min	ED_{50} Potency Ratio to (+)-Tubocurarine ($1/N$)	Slope Comparison[b] [$F_S(dF', dF)$]	Previous Work[c]
(+)-Tubocurarine	0.73	1.32	35	1		
XIII	0.85	17.06	20	13	0.018 (2, 27) +	
XIV	0.80	27.72	20	20		
IX	0.51	16.98	11	13	0.571 (2, 30) +	
X	0.66	38.10	9	29		
XI	0.94	41.41	15	31	0.295 (2, 27) +	(32–34) − (35) +
XII	0.80	47.67	15	36		
III	0.69	49.09	30	37	1.511 (2, 30) +	
IV	0.78	85.92	30	65		
VII	0.85	79.48	8	60	9.515 (2, 27) −	(36) − (37) +
VIII	0.75	128.97	7	97		
I	0.72	96.99	12	73	1.602 (3, 46) +	(9, 10, 38, 39) +
II	0.79	167.94	15	127		
V	0.92	260.88	10	197	3.073 (2, 27) +	
VI	0.86	488.98	9	370		

[a] When the raw data for a single animal were treated statistically, r^2 was considerably higher (>0.90) in all cases. The lower r^2 value obtained when the data for three cats were pooled can then be taken as an expression of individual variation. [b] Comparisons were made between the regression line slopes of the probes and (+)-tubocurarine (+ indicates that the slopes are not statistically dissimilar; — indicates that the slopes are statistically dissimilar). [c] Reference to prior studies on the racemic modifications (+ indicates potency agreement; — indicates potency disagreement).

Table II—Enantiomeric Neuromuscular Junction Blocking Potency Comparisons

Drug	Enantiomer	ED_{50} with 95% Confidence Limits, mg/kg			ED_{50} Potency Ratio of Enantiomers	Elevation[a] Comparison [$F_{el}(dF', dF)$]
		Low	ED_{50}	Up		
(+)-Tubocurarine		0.08	0.09	0.16		
I	(*S*)	3.29	4.84	11.68	1.8:1	3.198 (1, 25) −
II	(*R*)	5.24	8.38	20.56		
III	(*S,S*)	1.64	2.44	4.28	1.7:1	6.939 (1, 19) +
IV	(*R,R*)	3.43	4.27	5.86		
V	(*S*)	8.39	9.47	11.07	1.9:1	18.267 (1, 16) +
VI	(*R*)	13.39	17.75	24.70		
VII	(*S*)	3.58	3.95	4.36	1.7:1	17.240 (1, 16) +
VIII	(*R*)	5.07	6.41	8.00		
IX	(*S*)	0.29	0.82	1.64	2.2:1	7.992 (1, 19) +
X	(*R*)	1.30	1.84	3.33		
XI	(*S*)	1.70	2.00	2.40	1.2:1	−
XII	(*R*)	1.70	2.32	4.35		
XIII	(*S*)	0.61	0.80	1.22	1.5:1	4.563 (1, 16) +
XIV	(*R*)	0.90	1.30	1.50		

[a] Statistical comparisons were made between the line elevations of enantiomeric pairs to evaluate the significance of the difference in ED_{50} values (+ indicates a statistically significant difference; — indicates that the difference is not statistically significant).

probes showed identical, characteristic stepwise patterns of recovery when edrophonium, an acetylcholinesterase inhibitor, was administered (Fig. 1).

5. A statistical comparison of the slopes of log dose *versus* probit response linear regression lines (Fig. 2) for (+)-tubocurarine and the probes revealed that, in all cases except for the glauciniums, their slopes were not statistically different. This finding in itself is taken to mean a similar mechanism of action.

Table I presents the neuromuscular junction blocking potency data and is arranged so that the more potent inhibitors are at the top of the table. The phenolic aporphiniums are more potent than the aporphiniums which possess only aryl ether type of functional-

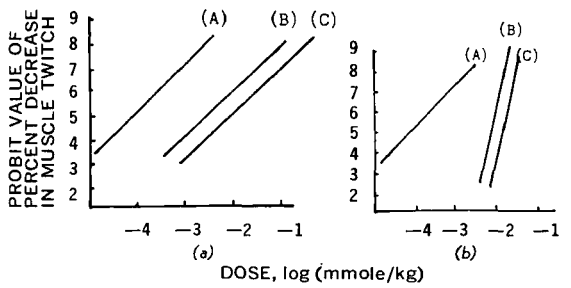


Figure 2—*Computer program-derived linear regression plots of neuromuscular junction blockage data.* (a) *Typical data represented by actual data for the* N-*methylcorydinium iodides. Key: A,* (+)-*tubocurarine; B, IX; and C, X.* (b) *Aberrant data obtained for the* N-*methylglaucinium iodides. Key: A,* (+)-*tubocurarine; B, VII; and C, VIII.*

ity, although it is usually expected that alkylation of phenolic groups leads to an enhancement in neuromuscular junction blocking potency (5, 40). However, the fully aryl ether type of aporphiniums are represented only by the glauciniums and, as already stated, these probes are unique in that their slopes show a statistically significant deviation from the slope of (+)-tubocurarine.

In addition to the neuromuscular junction activity, an initial fall in blood pressure was observed intermittently for the probes and for (+)-tubocurarine. The effect is common (41) for quaternary-type compounds and may be attributed to either a weak muscarinic-type activity or, more likely, to an initial histamine release effected by these agents since the effect was of a transient nature.

Another observation was that during the initial dosing phase with (+)-tubocurarine, equal subsequent doses effected a greater blockade. This phenomenon is presumed to be due to the filling of other more generalized receptors for quaternary-type compounds. The possibility that these receptors may not be located at the neuromuscular junction is exemplified by the red blood cells in which the membrane contains cholinesterase and bears a net negative charge (42, 43). After saturation of these depots with either (+)-tubocurarine or the probes, the phenomenon was no longer observed and linear relationships between dose and response could be obtained through the remainder of the experiment.

Because of this phenomenon, it became important to establish that these probes had an activity of their own and that they were not acting by the mere displacement of (+)-tubocurarine from the nonspecific receptors. Therefore, the Latin-square method was adopted into the drug administration protocol such that each new animal preparation was titrated and initially tested with a different drug. Three probes were tested in three different animal preparations, and each probe was tested with at least three different concentrations in each animal. Potency ratios were found to be the same in all preparations and were independent of the order of testing.

Table II presents the neuromuscular junction blocking potency

Table III—Enantiomeric Probe Binding to Blood Components

Drug	Test Concentration, mg/ml	Percent Bound to Plasma Proteins	Percent Bound to Red Blood Cells
I	0.085	9 ± 2	22 ± 2
II	0.085	8 ± 2	10 ± 2
III	0.040	9 ± 3	7 ± 2
IV	0.040	7 ± 3	10 ± 2
V	0.170	2 ± 3	—
VI	0.170	4 ± 3	—
VII	0.065	33 ± 8	12 ± 2
VIII	0.065	20 ± 6	13 ± 2
IX	0.016	50 ± 6	—
X	0.016	52 ± 4	—
XI	0.028	69 ± 7	22 ± 2
XII	0.028	59 ± 7	16 ± 2
XIII	0.013	68 ± 7	—
XIV	0.013	56 ± 6	—

relationships between the *(S)*- and *(R)*-enantiomers. That the *(S)*-enantiomers have a subtle, statistically significant potency magnitude difference from the *(R)*-enantiomers is clearly demonstrated. However, this difference reflects the gross response observations from an *in vivo* bioassay and cannot, at this point, be attributed to a stereochemical preference exhibited by certain neuromuscular junction receptors. Other physiological parameters that could cause such differences are: different absorption rates between enantiomers, unequal distributions due to either a preferential access to certain compartments or a preferential binding in certain compartments having equal accessibility, different metabolic rates, differing rates of excretion, and, most important, different inhibitory interactions with acetylcholinesterase. Since acetylcholinesterase is so intimately associated with the neuromuscular junction, its consideration is essential for studies of this type, even by those workers who employ isolated nerve–muscle preparations as their bioassay.

The time course of less than 5 sec to obtain maximal blocking effect after intravenous injection with all compounds tested represents very nearly the bolus head of the injection (44); on this basis, differential absorption, excretion, and metabolism rates are unlikely factors to be considered in seeking causes for differences in enantiomeric potency. Similarly, preferential access to certain compartments can probably be disregarded, although preferential binding (*e.g.*, to plasma protein) in certain compartments having equal accessibility cannot be dismissed as a possibility. Finally, preferential inhibition of acetylcholinesterase by one of the enantiomers certainly cannot be disregarded on this basis and, therefore, requires further investigation.

That the blood compartment may have binding preferences for one of the enantiomeric species was a primary consideration, al-

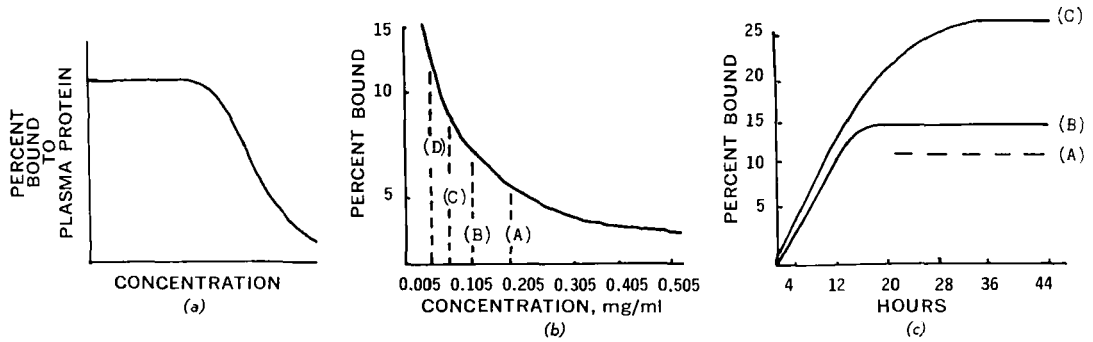


Figure 3—*Plasma protein binding patterns and* in vivo *drug distribution compartment concentrations.* (a) *Theoretical pattern of binding to plasma proteins as a function of increasing drug concentration.* (b) *Binding pattern observed within the measurable concentration range for the probes (actual data for I). Also illustrated are* in vivo *distribution compartment concentrations using the plasma volume (A), the total blood volume (B), the total extracellular water (C), and the total body water (D).* (c) *Typical dialysis rate data (actual data for I). Key: A, calculated loss due to dilution; B, control dialysis with membrane containing buffer solution only; and C, normal dialysis.*

Table IV—Acetylcholinesterase Inhibition

Drug	Enantiomer	*In Vivo* Concentration Range, $\times 10^{-5}$ M[a]	Wilkinson K_i, $\times 10^{-4}$ M[b]	Wilkinson V_{max}, μmoles/unit/min	Inhibition[c]
Acetylcholine (natural substrate, K_m)			2.72 ± 0.06	1.07 ± 0.01	
Edrophonium			0.033 ± 0.001	0.7 ± 0.2	
Neostigmine			0.0011 ± 0.0003	1.1 ± 0.4	
I	(*S*)	2–6	13 ± 0.2	1.1 ± 0.1	C
II	(*R*)	4–10	6.5 ± 0.8	1.0 ± 0.1	C
III	(*S,S*)	2–6	7.1 ± 0.4	0.8 ± 0.2	C
IV	(*R,R*)	4–10	6.1 ± 0.5	0.7 ± 0.3	C
V	(*S*)	13–32	8.2 ± 0.4	1.4 ± 0.3	C
VI	(*R*)	24–60	3.4 ± 0.3	1.19 ± 0.08	C
VII	(*S*)	4–10	1.7 ± 0.3	0.5 ± 0.1	I
VIII	(*R*)	6–16	2.3 ± 0.4	0.5 ± 0.04	I
IX	(*S*)	0.8–2	3.4 ± 0.2	1.4 ± 0.3	C
X	(*R*)	2–5	6.7 ± 0.3	1.1 ± 0.2	C
XI	(*S*)	2–5	3.9 ± 0.8	1.1 ± 0.3	C
XII	(*R*)	2–6	2.8 ± 0.5	1.6 ± 0.5	C
XIII	(*S*)	1–2	1.8 ± 0.4	0.3 ± 0.2	I
XIV	(*R*)	1–3	1.0 ± 0.3	1.0 ± 0.3	I

[a] Lower concentrations were calculated using the total body water as a distribution compartment, and upper concentrations were calculated using the total extracellular water as a distribution compartment. [b] Determined by calculation from computer program-(Wilkinson) generated, altered K_m values. [c] Determined graphically by Lineweaver–Burk plot (C = competitive and I = inconclusive).

though differences in enantiomeric activities on this basis are not common; a rather high degree of binding to the blood components is required before significant alterations in drug distribution patterns are observed (45). Nevertheless, two blood components may be entertained as potential binding sites, namely, plasma proteins and the red blood cells, since both have been implicated to some degree in previous studies (43, 46).

Plasma protein binding studies, utilizing a membrane equilibrium dialysis technique (47) and UV spectrophotometric measurement for concentration assay, were undertaken first. Preliminary studies had established that a UV assay was appropriate in terms of sensitivity in the concentration ranges expected and because the probes showed a conformity to the Beer–Lambert law. Figure 3 illustrates the nature of the results obtained. Failure to obtain the percent bound plateau at low concentrations as predicted by theory (48) is readily explained by the inability of UV spectrophotometry to assay at appropriately low concentrations for the agents employed as probes in this work. However, the concentration range that has been successfully studied is well below the range (Fig. 3) regarded as a minimum for an *in vivo* ED_{50}-based concentration range (44).

Furthermore, since the objective of the study was to compare the differences in binding between *(S)*- and *(R)*-enantiomers under identical, simulated *in vivo* conditions, whether or not measurements were made at the theoretical plateau levels became an academic question. Therefore, the concentrations employed were calculated from the average of the ED_{50} doses of the enantiomeric pair using the whole blood volume (44) as an appropriate *in vivo* distribution compartment (45). The results of the plasma protein binding study are recorded in Table III and clearly indicate that there are no significant differences in binding of the enantiomers; therefore, this type of interaction cannot account for the differences in neuromuscular junction blocking potency.

Aside from the question of enantiomeric binding comparisons, it was observed that there is a significantly greater binding propensity among the phenolic probes than among the nonphenolic ones. Since binding is a function of concentration in the range studied, Table III cannot be used for such a comparison. However, Fig. 4 shows this difference clearly. This behavior is in accord with observations of earlier workers (46) who found similar binding enhancement in the presence of hydrogen-bonding phenolic or hydroxyl groups.

Binding to red blood cells was studied next. Four of the seven pairs of enantiomers under consideration were selected for the study and represent all of the structural types present among the probes. Thus, I and II were representative of the tetrahydroisoquinoliniums, III and IV were representative of the *N*-methylpaviniums, and VII and VIII and XI and XII were representative of the nonphenolic and phenolic aporphiniums, respectively. Dialysis, in this case, was not necessary since centrifugation could remove the red blood cells from the sample and produce a clear solution suitable for UV analysis.

The same concentrations used in the plasma protein binding study were employed in determining the extent of binding to the red blood cells (Table III). While statistically different binding is observed for the enantiomeric cases of I and II and XI and XII, it would be unreasonable to consider these to have a causal relationship with the observed *(S)*- greater than *(R)*-enantiomeric neuromuscular junction potency differences because the result [*(S)* bound greater than *(R)*] is opposite to what would be required.

Preferential inhibition of acetylcholinesterase by one of the enantiomers, which would presumably tend to offset the neuromuscular junction blocking activity by that enantiomer and thereby result in a greater apparent activity for the other enantiomer, was the final possibility studied. The pH–stat method (49–52) was chosen. In this method, the acetic acid produced by enzymatic hydrolysis of acetylcholine is automatically titrated with base so that the pH is maintained at 7.5.

The enzymatic velocity so obtained was treated by a computer analysis[2], using the method of Wilkinson (53), to generate K_m and V_{max} data having standard deviation error limits associated with each value. From these data, the K_i values could be calculated by employing simple Michaelis relationships[3]. Lineweaver–Burk plots (54) were also performed to aid in the assessment of the type of inhibition exhibited by these agents.

The results of these studies are recorded in Table IV. The K_m and V_{max} data for acetylcholine indicate that the method is both valid [the K_m being in general agreement with literature values (49–51, 55) and the V_{max} being in exact agreement[4]] and highly accurate (the K_m error limit being only ±2% and that for V_{max} being only ±1%). The expansion of these error limits when the inhibitors are present implies that the interaction of these agents with the enzyme is more complex than that dictated for narrow error range limits by the simple mathematical model used in the data regression technique. However, the values are accurate enough in that they distinguished enantiomeric potency differences in five of the seven probe pairs. Also, the agreement between the Wilkinson V_{max} data and the Lineweaver–Burk data strengthens the validity of the generated data.

As expected (56–59), the results indicate that the probes are,

[2] CDC-6400 computer program.

[3]
$$\frac{X \text{ intercept}}{\text{altered } X \text{ intercept}} = 1 + \frac{[I]}{K_i}$$

[4] One unit of acetylcholinesterase hydrolyzes 1 μmole of acetylcholine/min at 25° (Worthington Biochemical Corp.).

Table V—Racemic Bases

Compound	Preparation Reference	Aporphine Racemization Time, hr	Overall Yield, %	Melting Point	Literature Melting Point
(±)-Laudanosine	12		83	114–116°	115–116° (60)
[(±)-1-(3′,4′-dimethoxybenzyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline]				172–174° (picrate) 214–216° (methiodide)	174–175° 215–217°
(±)-Salsolidine	20		35	201–202° (picrate)	201–201.5° (61)
[(±)-1,2-dimethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline]					
(±)-*N*-Methylpavine	17		20	134–138°	139–140° (18)
[(±)-2,3,8,9-tetramethoxy-*N*-methylpavinane]	18				
(±)-Glaucine	21	36	51	134–137°	137–139° (60)
[(±)-1,2,9,10-tetramethoxyaporphine]	22				
(±)-Corydine	22	72	75	164–166° 226° (·HCl)	165–167° (62) 228° (63)
[(±)-1-hydroxy-2,10,11-trimethoxyaporphine]					
(±)-Isocorydine	22	125	56	120° (amorphous solid)	
[(±)-1,2,10-trimethoxy-11-hydroxyaporphine]					
(±)-Boldine[a]	22	125	78	86° (amorphous solid)	
[(±)-1,10-dimethoxy-2,9-dihydroxyaporphine]					

[a] If the boldine or boldinium-type compounds were left standing in the open for several days, decomposition would occur. Aqueous solutions were even less stable and darkened after a single day. This behavior is in accord with the literature (60).

mainly only very weak inhibitors of the enzyme, since they show K_i values larger than the K_m value for acetylcholine. The only two exceptions are the glauciniums (VII and VIII) and the boldiniums (XIII and XIV), whose K_i values are slightly less than the K_m value for acetylcholine, but they are still regarded as only weak inhibitors. In general, the nature of this inhibition would appear to be largely competitive, as shown by the insignificant alteration of the Wilkinson V_{max} data and by Lineweaver–Burk plot assessment. Again, the glauciniums and boldiniums are exceptions and probably have a different or mixed mechanism of action.

The K_i values are generally larger than the appropriate *in vivo* neuromuscular junction ED_{50} blocking concentrations by a factor of nearly 10. This weak inhibitory activity, when coupled with *in vivo* concentration considerations, indicates that the action of the probes on acetylcholinesterase can contribute only insignificantly to the overall neuromuscular junction blocking activity observed as the gross response to these agents. Nevertheless, such small enzyme interactions possibly could be enough to distort the gross neuromuscular junction responses between the *(S)*- and *(R)*-enantiomers if the *(S)*- and *(R)*-enantiomer interactions with the enzyme show substantial differences.

An assessment of this possibility requires a specific examination of Table IV in terms of *(S)*- and *(R)*-enantiomeric differences. Statistically, an *(R)* > *(S)* potency relationship exists in four cases, *(S)* > *(R)* in one case, and *(S)* = *(R)* in the remaining two cases. Clearly, the definite trend in gross neuromuscular junction blocking potency, where the *(S)*-enantiomers are always more potent than the *(R)*-enantiomers, is not so unequivocally approached in the anticholinesterase activity series. Therefore, it does not seem reasonable to equate the gross enantiomeric differences with some causative relationship involving antiacetylcholinesterase enantiomeric potency differences.

Furthermore, for one of the enantiomeric pairs (I and II) showing the most substantial difference in inhibitory potency, the difference in gross neuromuscular junction blocking potency is not even statistically significant. Similarly, for the enantiomeric pair (IX and X) showing the largest gross neuromuscular junction blocking potency difference, the inhibitory difference is in the opposite direction [*(S)* > *(R)*] to that required for the production of the gross neuromuscular junction response difference if the difference is to be attributed to the difference in enzyme interactions. Both of these findings serve to negate further the notion that there is a causal relationship between antiacetylcholinesterase enantiomeric potency differences and gross neuromuscular junction blocking enantiomeric potency differences.

All of the evidence, therefore, implies that the inhibitory activity is of too low a potency and is too random in nature to result in the observed unequivocal relationship between the *(S)*- and *(R)*-enantiomers in gross neuromuscular junction blocking potency.

EXPERIMENTAL[5]

Racemic Bases—The preparation of the individual racemic laudanosine, salsolidine, and *N*-methylpavine bases was performed according to conventional published methods, although (+)-glaucine[6], (+)-corydine[7], (+)-isocorydine[7], and (+)-boldine[8] were obtained from commercial sources. The latter four compounds were racemized by the method of Kametani *et al.* (22), using Adam's catalyst under catalytic hydrogenation conditions.

Table V summarizes the overall yields, physical constants, and references for the preparative procedures employed. Trivial names, historically assigned, are used for convenience, together with a statement of the more complex systematic name.

Resolution of Racemic Bases—Deracemization was accomplished through the formation of diastereomeric bitartrate salts obtained through interaction of the racemic base with the appropriate (+)- or (−)-tartaric or substituted tartaric acid. With racemic laudanosine, salsolidine, and *N*-methylpavine, it was necessary to use both the (+)- and (−)-forms of the tartaric acids; with the commercially obtainable, optically active bases, it was necessary to use only one of them.

The purity of the resolved bases was maintained at a high level. Table VI summarizes the yields and physical constants for the enantiomeric pairs and indicates the deracemization techniques employed.

[5] Melting points were determined on a Thomas-Hoover melting-point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Analyses were performed by M-H-W Laboratories, Garden City, Mich. TLC was conducted on Eastman Chromagram (No. 6060) sheets in a minimum of three different solvent systems, and visualization was done with both UV lamp and iodine vapor. IR spectra were obtained as KBr samples with either a Perkin-Elmer 237 B grating IR spectrometer or a Beckman IR 9 spectrometer. UV spectra were determined on a Cary 14 recording spectrophotometer. NMR spectra were measured with a Varian Associates model A-60D or T-60 NMR spectrometer, using tetramethylsilane as an internal standard. Mass spectral determinations were performed by Mass Spectrometry Laboratory Services, Department of Chemistry, University of Minnesota, Minneapolis, Minn., using a AEI-MS 30 mass spectrometer or a Hitachi Perkin-Elmer RMU-6D instrument.

[6] Pierce Chemical Co., Rockford, Ill.

[7] Obtained many years ago from Eastman Chemical Co., Rochester, N.Y.

[8] Nutritional Biochemicals Corp., Cleveland, Ohio.

Table VI—Enantiomeric Bases

Compound	Resolution Reference	Resolving Acid (Solvent)	Yield, %	Melting Point	$[\alpha]_D^{28}$	Literature Melting Point	$[\alpha]_D$
(+)-(*S*)-Laudanosine	13	*O,O*-Di-*p*-toluoyltartaric [ethyl acetate–acetone (7:3)]	82	109–110°	55.4 (c 1, $CHCl_3$)	109° (13)	56.8 ($CHCl_3$)
(−)-(*R*)-Laudanosine		—	54	110°	−55.2 (c 1, $CHCl_3$)	109° (13)	−56.8 ($CHCl_3$)
(+)-(*R*)-Salsolidine	21	*O,O*-Dibenzoyltartaric (ethanol)	35	Liquid	60 (c 1, C_2H_5OH)	—	60.3 (C_2H_5OH) (21)
(−)-(*S*)-Salsolidine		—	40	Liquid	−59 (c 0.5, C_2H_5OH)	—	−59.9 (C_2H_5OH) (61)
(+)-(*R,R*)-*N*-Methylpavine	19	Tartaric acid (ethanol)	64	151–153°	209 (c 1, C_2H_5OH)	151–153° (19)	207.8 (C_2H_5OH)
(−)-(*S,S*)-*N*-Methylpavine		—	66	151–152°	−209 (c 1, C_2H_5OH)	152–153° (19)	−209 (C_2H_5OH)
(+)-(*S*)-Glaucine[a]	23	None[b]	—	118–120°	115 (c 0.5, C_2H_5OH)	119–120° (63)	115 (C_2H_5OH)
(−)-(*R*)-Glaucine		(+)-Tartaric (ethanol)	56	Oil[c]	−115 (c 0.5, C_2H_5OH)	—	−115 (C_2H_5OH) (23)
(+)-(*S*)-Corydine[a]	62	None[b]	—	147–149°	204 (c 2, $CHCl_3$)	149° (62)	204 ($CHCl_3$) (63)
(−)-(*R*)-Corydine		(+)-Tartaric (water)	45	146–148°	−205 (c 2, $CHCl_3$)	149° (62)	−206 ($CHCl_3$)
(+)-(*S*)-Isocorydine[a]	—	None[b]	—	182–184°	194 (c 0.5, $CHCl_3$)	185° (63)	195 ($CHCl_3$)
(−)-(*R*)-Isocorydine		(+)-Tartaric (50% aqueous ethanol)	15	Oil	−194 (c 0.5, $CHCl_3$)	—	—
(+)-(*S*)-Boldine[a,d]	—	None[b]	—	160–163°	112 (c 0.5, CH_3OH)	161–163° (63)	114 (C_2H_5OH)
(−)-(*R*)-Boldine[d]		(+)-*O,O*-Di-*p*-toluoyl-(−)-tartaric (ethyl acetate–acetone)	24	160–163°	−111 (c 0.5, CH_3OH)	—	

[a] Physical data and literature values were obtained and are reported for these commercially available, naturally occurring (*S*)-enantiomers for the purpose of comparison with the unnatural (*R*)-enantiomers obtained by deracemization. [b] Naturally occurring alkaloids not requiring deracemization and obtained from commercial sources. [c] All other physical and spectral data were in accord with the literature values. Since the next preparative step involved simple nitrogen quaternization, the pursuit of crystalline material seemed more reasonable after this next reaction step; extraordinary attempts to produce crystalline material were not pursued at this stage. [d] Literature data and the values obtained in this work are for boldine obtained from chloroform solutions.

Table VII—Probe Compounds

Compound	Yield, %	Melting Point	$[\alpha]_D^{28}$	Literature Melting Point	$[\alpha]_D$
I	90	218–220° dec.	118 (c 1, C_2H_5OH)	218–221° dec. (64)	120 (C_2H_5OH)
II	90	216–219° dec.	−117 (c 1, C_2H_5OH)	218–221° dec.	−120 (C_2H_5OH)
III	91	273–276°	−204 (c 0.5, 50% aq. CH_3OH)	270–280° (60)	—[a]
IV	91	272–276°	204 (c 0.5, 50% aq. CH_3OH)	270–280°	—[a]
V	68	198–202° dec.	10.6 (c 1, C_2H_5OH)	211° dec. (in a sealed tube) (63)	—[a]
VI	52	196–200° dec.	−10.7 (c 1, C_2H_5OH)	211° dec. (in a sealed tube)	—[a]
VII	69	220–222° dec.	78 (c 0.5, C_2H_5OH)	224–225° (60)	72.5
VIII	65	223–224° dec.	−77 (c 0.5, C_2H_5OH)	224–225°	−72.5
IX	64	212–214° dec.	136 (c 0.8, $CHCl_3$)	228–230° dec. (62)	—[a]
X	43	214–216° dec.	−138 (c 0.8, $CHCl_3$)		—[a]
XI	75	239° dec.	148 (c 0.3, H_2O)	231–232° (rapid heating) (60)	139.9 (62) 143.3
XII	70	236° dec.	−146 (c 0.5, H_2O)		—[a]
XIII	31	178–182°	5.4 (c 0.5, CH_3OH)	"...boldine...does not yield crystalline salts" (62)	—[a]
XIV	28	180–182°	−5.2 (c 0.5, CH_3OH)		—[a]

[a] Since optical rotation data were not found in the literature for these compounds, their analyses are reported in Table VIII.

Table VIII—Elemental Analyses

Probe	Formula	Analysis, % Calculated C	H	I	N	Found C	H	I	N
III	$C_{22}H_{28}INO_4 \cdot 2H_2O$	49.54	6.02	23.84	2.63	49.70	5.88	23.64	2.50
IV						49.67	5.84	23.96	2.55
V	$C_{14}H_{22}INO_2$	46.28	6.06	34.99	3.86	46.38	5.82	34.82	3.66
VI						46.16	5.85	35.16	3.69
IX	$C_{21}H_{26}INO_4 \cdot 2H_2O$	48.55	5.78	24.47	2.69	48.10	5.43	23.88	2.50
X						48.65	5.77	24.67	2.48
XII	$C_{21}H_{26}INO_4$	52.17	5.38	26.29	2.90	52.07	5.13	26.10	2.79
XIII	$C_{20}H_{24}INO_4$	51.13	5.11	27.06	2.98	51.09	4.99	26.89	2.77
XIV						51.10	4.85	27.33	2.76

Quaternization of Enantiomeric Bases—Simple treatment of a methanolic solution of the base with excess methyl iodide was the general procedure. In the case of the salsolidines (secondary amines), it was necessary to buffer the reaction carefully with sodium bicarbonate to take up the elaborated hydrogen iodide to permit complete quaternization.

Table VII records the yields and physical constants for these final compounds. Elemental analyses for previously unreported compounds are recorded in Table VIII.

Neuromuscular Junction Blocking Bioassay—Male cats, 2.25–4 kg, were anesthetized by intraperitoneal injection of either sodium pentobarbital or allobarbituric acid. The femoral artery and vein were exposed by surgery and cannulated. The arterial cannula was connected to a force transducer[9], which was coupled[10] to a recording polygraph[11]. An accurate tracing of the animal's blood pressure was thus obtained and used to ascertain the general condition of the specimen and the depth of anesthesia. Finally, it was used for verification of death which was effected with intravenous administration of a saturated potassium chloride solution at the completion of the experiment.

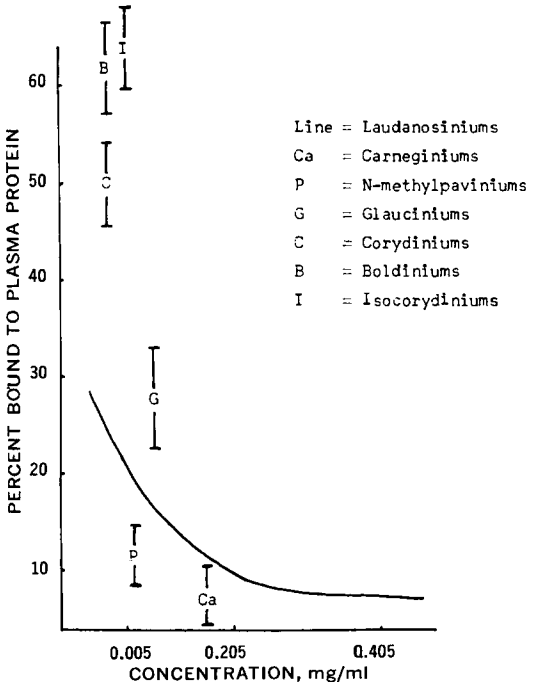


Figure 4—*Relationship of protein binding between different drug families.*

The venous cannula, intended for drug administration, led to a three-way stopcock, which allowed very small volumes of probe solutions to be flushed into the animal *via* another larger syringe containing normal saline. A tracheotomy was performed and the animal respired comfortably with the assistance of a respirator[12] (16–20 strokes/min; 20–30 ml/stroke). The hypoglossal nerve was surgically exposed, and an electrode was placed under the nerve so that a supramaximal (1–5 v) square (1-msec duration, 4.5-sec delay) pulse was given by an electrical stimulator[13]. The tongue muscle was tied with surgical thread leading to a strain-gauge transducer[14] and, *via* a coupler[10], allowed for muscle-twitch–pen-flick recording on the polygraph (Fig. 1). All drug solutions were prepared in normal saline, and the administration protocol followed the Latin-square method described previously.

Data Regression—Raw data, as percent decrease from control response, were converted to probit values. These values *versus* log dose values were used in a linear regression program to generate computer-derived lines having 95% confidence limits associated with their error parameters. From the line and error parameters, the following computer programmed calculations were performed: an ED_{50} with 95% confidence limits, a statistical comparison of the slopes between the different drugs, and a comparison between the ED_{50} values in an indirect manner *via* a statistical comparison of the line elevations between different drugs.

Plasma Protein Binding Assay—The equilibrium dialysis setup was identical to that described by Abdel-Monem *et al.* (47). Plasma proteins were obtained from cat whole blood by using the supernate after centrifugation (2000×*g* for 20 min at 25°). All solutions were prepared from isotonic pH 7.4 phosphate buffer solution; the drug concentrations are specified in Table III.

UV methods were used for the assay of probe concentration in the external medium. Appropriate control runs were performed to account for drug losses from the external medium due to simple dilution and dialysis membrane binding (Fig. 3). Dialyses were run for 48 hr (double the time required for equilibrium). The concentration assay was performed before and after dialysis and the difference, after accounting for dilution and membrane binding losses, was used to calculate the percent bound to plasma proteins. Each drug was studied in triplicate, and the data were treated as an average value plus or minus an error limit, which would account for 100% of the data.

Red Blood Cell Binding Assay—Red blood cells were obtained from cat whole blood by four repetitions of centrifugation (2000 × *g* for 20 min at 25°), washing with isotonic pH 7.4 phosphate buffer solution, and, finally, dilution with buffer solution to approximately the same volume of whole blood from which they were obtained. The red blood cell solutions and drug solutions were equilibrated for 8 hr (double the time required for equilibration); then the red blood cells, with bound drug, were removed by centrifugation. The probe concentrations employed are specified in Table III.

UV assays were used to determine probe concentration before

[9] Beckman 215071.
[10] Beckman type 9853 coupler.
[11] Beckman type R-411 dynograph.
[12] Harvard apparaus respirator for small animals.
[13] Grass S44.
[14] Statham model UC-3.

and after equilibration. The difference, after accounting for dilution effects, was used to calculate the percent of probe bound to the red blood cells. Each probe was studied in triplicate, and the data were treated in exactly the same manner as for the plasma protein binding study.

Acetylcholinesterase Inhibition Studies—The pH–stat method was previously described (49–52). Experiments were performed with an automatic titrator[15] with recording features. Each run employed 0.35 unit[4] of eel acetylcholinesterase[16]. Five concentrations of acetylcholine chloride[17], varying from 1.0 to 5.0 × 10^{-4} *M*, were used as substrate. The titrant solution was 0.0005 *N* NaOH solution.

Three inhibitor concentrations were used with at least four substrate concentrations so that a minimum of 12 runs was performed for each probe. Inhibitor concentrations were in the range of 2–15 × 10^{-4} *M* and were such that an observable decrease in enzymatic velocity was obtained. All solutions were prepared from doubly distilled deionized water and contained 0.02 *M* $MgCl_2$ and 0.1 *M* NaCl. The enzyme solution also contained 0.005% bovine serum albumin.

All runs were performed at 28°. Aqueous hydrolysis of acetylcholine (51) was found to be insignificant, as was the need for a stream of nitrogen (50) in the reaction vessel. The data regression is described under *Biology*.

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[15] Copenhagen Radiometer automatic titrator setup employing a type SBR 2C titrigraph, a type TTT2 titrator, and a type ABU 12 autoburet.

[16] Product No. 5310, code ECHP, Worthington Biochemical Corp., Freehold, N.J.

[17] Sigma Chemical Co., St. Louis, Mo.

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Stereochemical Preferences for Curarimimetic Neuromuscular Junction Blockade II: Enantiomeric Bisquaternary Amines as Probes

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Abstract □ Two pairs of bisquaternary enantiomeric neuromuscular junction blocking agents as well as their diastereomeric *meso*-forms were prepared in which the carbon asymmetry is adjacent to the quaternary center. The tertiary amines from which the blocking species were obtained by methyl iodide treatment were *N*-methylpavine and 1,1′-dodecamethylenebis(6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline). Blocking potencies were determined by the mouse inclined screen assay and by the cat tongue–hypoglossal nerve technique. The mouse assay showed no statistical difference between the enantiomeric probes derived from *N*-methylpavine and only a modest superiority of the *(R–R)* isomer over the *(S–S)* isomer in the case of the tetrahydroisoquinoline compounds. The cat assay showed a modest statistically significant *(R–R)* > *(S–S)* difference in potencies in both kinds of probes. The diastereomeric *meso*-compounds were less active than the enantiomers in mice but were of intermediate activity in the cat determination. Acetylcholinesterase-inhibiting activity was determined for each probe to discount potency differences from this source, and no significant differences in blocking potency attributable to preferential enzyme inhibition by the probes were noted.

Keyphrases □ Curarimimetic neuromuscular junction blockade—stereochemical preferences, enantiomeric bisquaternary amines as probes □ Neuromuscular junction blockade, curarimimetic—stereochemical preferences, enantiomeric bisquaternary amines as probes □ *N*-Methylpavine (enantiomeric bisquaternary amines)—probes for stereochemical preferences for curarimimetic neuromuscular junction blockade □ 1,1′-Dodecamethylenebis(6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (enantiomeric bisquaternary amines)—probes for stereochemical preferences for curarimimetic neuromuscular junction blockade

The initial report (1) from these laboratories concerning the possibility of stereochemical preferences being exhibited at the neuromuscular junction toward nondepolarizing blocking agents of the curare type was concerned with several monoquaternary enantiomeric probes derived from alkaloids related to tetrahydroisoquinoline. These studies examined the possibility that the exclusive, but modest, *(S)* > *(R)* (about 1.8:1) blocking potency difference shown by the cat assay could have been due to stereopreferential binding of the probes by blood components and/or by stereoselective acetylcholinesterase inhibition since other factors such as absorption, excretion, and metabolism differences were unlikely due to the rapid onset of block. The blood binding studies on both plasma protein and red blood cells indicated a low order of binding and a reversed order from that which might account for potency differences. The stereoselective inhibition of acetylcholinesterase, while it had the correct orientation in some cases, was of such a low order of activity and so random in its focus that it could not be seriously considered as causal for the observed potency differences.

DISCUSSION

Since neuromuscular junction blockers have traditionally been thought of as bisquaternaries, in spite of the recent disclosure that (+)-tubocurarine is actually a monoquaternary (2), it seemed appropriate to test neuromuscular junction stereochemical preferences on these types to see whether the preferences determined for monoquaternaries extended to the bisquaternaries. Unpublished observations in these laboratories had shown that quaternization of (±)-*N*-methylpavine (I) with 1,10-diiododecane produced a potent neuromuscular junction blocking agent (II) comparable in activity to (+)-tubocurarine. Therefore, the enantiomeric forms (II*a* and II*b*) of this quaternary blocker as well as the *meso*-form (II*c*) were considered to be suitable probes in the determination of stereochemical preferences at the neuromuscular junction for blocking agents.

Stereochemical preferences can only be considered valid when made between enantiomeric forms since diastereomers have different physical and chemical properties whereas enantiomers only differ in rotatory effect on polarized light. On the other hand, the fact that enantiomers may be operating in an asymmetric biological environment necessitates giving attention to preferential plasma protein and/or red blood cell binding as well as to stereopreferential acetylcholinesterase inhibition. Previous studies (1) demonstrated that blood components probably need not be considered as causal for differences in activity. However, acetylcholinesterase inhibition cannot be ruled out as a possible factor, even though it was inoperative in the case of the monoquaternaries, because it is