The Autonomic Margins of Safety of Metocurine and d-Tubocurarine in the Cat

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The potencies of metocurine and d-tubocurarine for neuromuscular and autonomic blockade and histamine release were determined in cats anesthetized with chloralose and pentobarbital. The autonomic margins of safety of these drugs were determined by measuring the ratios of EDₙₕ for sympathetic block to EDₙₕ for neuromuscular block; EDₙₕ for vagal block to EDₙₕ for neuromuscular block; EDₙₕ for histamine release to EDₙₕ for neuromuscular block. Metocurine is 14 times more potent than d-tubocurarine as a neuromuscular blocking agent in the cat, but its autonomic blocking action is three times weaker than that of d-tubocurarine and its histamine-releasing action is less than half that of d-tubocurarine. The combination of higher neuromuscular blocking potency and weaker autonomic effect gives metocurine a much higher autonomic margin of safety than d-tubocurarine in the cat. (Key words: Blood pressure; drug effects. Histamine. Neuromuscular relaxants: metocurine; d-tubocurarine. Parasympathetic nervous system: vagus. Sympathetic nervous system: ganglia.)

Metocurine, the N,N,O-trimethylated derivative of d-tubocurarine, formerly called dimethyltubocurarine, has considerably less tendency to produce hypotension in man than its parent compound.1–5 The hypotensive action of d-tubocurarine is known to occur because of its ability to inhibit transmission in autonomic ganglia and to release histamine.6 Several studies in animals have shown that the effects of metocurine on autonomic ganglia are weaker than those of d-tubocurarine.7–11 Metocurine's histamine-releasing activity is less than that of d-tubocurarine,8,10 but comparative potencies have not been established in dose—response fashion. Metocurine, on the other hand, is a more potent neuromuscular blocking drug in both man and animals than is d-tubocurarine.5,8,11 Therefore, the separation in dosages of the desired neuromuscular blocking action of metocurine from its side effects is much wider than that for d-tubocurarine. This investigation was designed to define the aforementioned interval, which we call the autonomic margin of safety, for metocurine and for d-tubocurarine. To do so, we determined the ED₉₅ for neuromuscular block and the ED₉₅ and mechanisms for inhibition of sympathetic and vagal (parasympathetic) transmission and for histamine release by metocurine and d-tubocurarine in the cat. The cat and man are generally considered to respond similarly to neuromuscular blocking agents,13 and the cat is commonly used to assay the autonomic effects of neuromuscular blocking drugs.7–11

Methods

Sixteen large adult cats of either sex weighing 3–5 kg were anesthetized with α-chloralose, 80 mg/kg, and pentobarbital, 7 mg/kg, given intraperitoneally. Each drug was studied in eight animals. Only one drug was studied in each animal. Cannulas were placed in the left femoral vein and artery for drug injection and for the recording of arterial pressure via a Statham P23DB transducer. Heart rate was measured by a Grass 7P44A tachograph triggered by the arterial pulse wave. The animals' lungs were mechanically ventilated through a tracheostomy by a Harvard small-animal ventilator set to deliver a 15 ml/kg tidal volume at 20 breaths/min. This ventilatory pattern resulted in end-tidal P₉₅ values in the range of 3–4 per cent, as indicated by a Beckman LB-1 gas analyzer.

The right vagus nerve and right sympathetic trunk were exposed and divided in the neck. The distal ends were placed on the same shielded platinum wire electrode to permit preganglionic stimulation of both nerve trunks. In most preparations the left sympathetic trunk was also dissected along its postganglionic portion at the base of the skull, and cut distal to the superior cervical ganglion to permit postganglionic stimulation through another electrode. Trains of square-wave pulses (20 Hz for 10 sec) were delivered at supramaximal voltage every 4 min simultaneously to all three autonomic nerve trunks by a Grass S-88 stimulator through an isolation unit. The resultant bradycardia and hypotension (the vagal response) were measured. Contractions of both nictitating membranes, one (the right) elicited preganglionically and the other (the left) elicited postganglionically, were recorded through Grass FT-03 transducers. In nearly all preparations the maximal vagal response (i.e., cardiac arrest for 10 sec) was achieved by stimulation

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of the right vagus alone. In the remainder, stimulation of both vagi simultaneously was necessary. When this was done, the left vagus was also divided in the neck.

Twitches of the right tibialis anterior muscle were elicited at 0.15 Hz via the peroneal branch of the right sciatic nerve, to which square-wave shocks 0.2 msec in duration derived from a Grass S88 stimulator and isolation unit were applied at supramaximal voltage. Twitch recording was done via a Grass FT-10 transducer. All nerves and tendons were kept moist in small pools of mineral oil or in cotton pledgets soaked in mineral oil. Tibialis anterior and esophageal muscle temperatures were monitored by Yellow Springs probes and kept between 35 and 38 C by heat lamps.

Simultaneous recordings of heart rate, arterial pressure, pre- and postganglionically elicited contractions of the nictitating membrane, and twitch of the tibialis anterior were made on a Grass Model 7 polygraph (fig. 1). Cumulative dose–response curves for inhibition of neuromuscular, vagal (parasympathetic) and sympathetic function were determined simultaneously for each animal. The mechanism of vagal inhibition was localized at parasympathetic ganglia or at cardiac muscarinic receptors by determining whether the bradycardic response to acetyl β-methyl choline (methacholine), 20 µg/kg, was blocked as well as the neurally-elicited bradycardia.7

The single-bolus dose of d-tubocurarine or metocurine producing the “delayed depressor response” described by Paton13,14 plus tachycardia was determined in each animal. The latter response is nearly pathognomonic of histamine release,13,14 and for the purpose of this study was defined as sudden hypotension to less than 80 percent of the control mean arterial pressure within 2 min of relaxant injection, with tachycardia to more than 25 percent above the baseline value. Further confirmation that the delayed depressor response was caused by release of histamine was obtained in each animal by determination of inhibition of the response by prophyaxis with the H1 and H2 receptor antagonists perphenazine, 1 mg/kg, and Metiamide, 1 mg/kg, given intravenously 20 min prior to dosage with metocurine or d-tubocurarine.

All drugs were given intravenously. Drugs used were metocurine iodide (Lilly), d-tubocurarine chloride (Abbot), acetyl β-methyl choline bromide (Aldrich), perphenazine (Scherling), and Metiamide hydrochloride (SKF). Dosages refer to the salts.

Data analysis was done by the method of Litchfield and Wilcoxon.15 Mean dose–response curves were plotted on log-probit paper. Best fit of the data to straight lines on these scales was determined by computerized regression. The cumulative ED50 values for vagal and sympathetic inhibition and the cumulative ED50 values for neuromuscular blockade were determined from the lines and 95 percent confidence limits were calculated. Goodness of fit of the data to straight lines (by χ2 test) and differences in potency were considered significant when P < 0.05.

The occurrence of histamine release was also treated as an all-or-none response, to permit log-probit plotting, in the following fashion. The delayed depressor response plus tachycardia was judged to have or to have not occurred after each single bolus dose of the drugs. The percentage of animals responding at each dose level was then determined and the data handled by the Litchfield–Wilcoxon method as described above.

The autonomic margins of safety of metocurine and d-tubocurarine were calculated as the ratios of the cumulative doses producing 50 percent block (ED50) of vagal (parasympathetic) and sympathetic transmission and the ED50 for histamine release, each divided by the ED50 for neuromuscular blockade.

Results

As dosage was increased, both d-tubocurarine (figs. 1A and 2A) and metocurine (figs. 1B and 2B) abolished the tibialis anterior twitch, prevented vagally-induced bradycardia, and decreased the amplitude of nictitating membrane contractions elicited preganglionically, i.e., produced neuromuscular, vagal, and sympathetic ganglionic blockade, in that order. Sympathetic inhibition by both d-tubocurarine and metocurine is clearly localized to the ganglia, since the preganglionically elicited contraction of the nictitating membrane was completely unaffected in either case (fig. 1A and B). Nonsignificant deviation from parallelism of the dose–response curves is supportive of similar mechanisms of sympathetic ganglionic block by the two compounds.

Vagal block by the drugs also appeared to occur principally at ganglia. The bradycardic response to acetyl β-methyl choline could still be elicited despite complete abolition of the response to vagus nerve stimulation by either d-tubocurarine or metocurine. Metocurine, however, also competitively antagonized the response to acetyl β-methyl choline at somewhat higher dosage, whereas d-tubocurarine did not. Therefore, muscarinic receptor blockade may contribute to the total vagolytic effect of metocurine. The latter contribution must be of minor importance, however, since the dose–response curve did not deviate significantly from parallelism with that of d-tubocurarine.

The data indicate that the autonomic effects of metocurine and d-tubocurarine are qualitatively sim-
Fig. 1. A, left, neuromuscular and autonomic pharmacology of \( d \)-tubocurarine in a 3.6-kg cat anesthetized with chloralose–pentobarbital. Time scale (minutes) at top. Simultaneous recording of (top to bottom) heart rate, femoral arterial pressure, post- and preganglionically elicited contractions of the left and right nictitating membranes, and right tibialis anterior twitch elicited via the peroneal nerve at 0.15 Hz. At each dot, stimulation of the right vagus nerve and both sympathetic trunks was carried out (20 Hz for 10 sec). At arrows, \( d \)-tubocurarine (\( d \)TC) was given intravenously in the dosage (mg/kg) indicated. The lower row of figures indicates cumulative dosage. Note the occurrence of neuromuscular block (lower record) closely followed within a narrow dose range by vagal inhibition (upper record) and sympathetic ganglion blockade (inhibition of preganglionically but not postganglionically-elicited contractions of the nictitating membrane). "V" between upper two records indicates points of vagal stimulation with little or no bradycardic response, i.e., the presence of high-degree vagal block. "H" indicates the occurrence of the "delayed depressor" response,\(^{10,11}\) indicating histamine release.

B, below, neuromuscular and autonomic pharmacology of metocurine in a 4.2-kg cat. Experimental protocol as in A. The separation of metocurine's autonomic actions from its neuromuscular blocking effect is much wider than the corresponding interval for \( d \)-tubocurarine (A).
Figure 2. A, above, cumulative dose–response curves for inhibition of neuromuscular and autonomic function by d-tubocurarine in the cat. Each point represents mean ± SEM of data from eight animals. Blockades of all responses occurred within a narrow dose range, indicating the well-known nicotinic blocking action of d-tubocurarine. Goodness of fit of the data to straight lines was significant ($P < 0.05$).

B, below, cumulative dose–response curves for inhibition of neuromuscular and autonomic function by metocurine in the cat. Each point represents mean ± SEM of data from eight animals. There was a wide separation between dosages producing neuromuscular and autonomic blocks. Goodness of fit of the data to straight lines was significant ($P < 0.05$).

ilar, but there are marked differences in the relative potencies of the two drugs. Metocurine is a significantly more potent neuromuscular blocking agent, and on the other hand, a significantly weaker inhibitor of ganglionic transmission, than d-tubocurarine (table I).

As the dose range producing sympathetic inhibition was achieved, both metocurine and d-tubocurarine produced tachycardia in addition to the delayed depressor response$^{13,14}$ (see fig. 1 A and B). The latter was markedly attenuated or completely inhibited by
prophylaxis with Metiamide and perphenazine. Both metocurine and \(d\)-tubocurarine, therefore, appear to release histamine at high dosages. The potency of metocurine in this respect, however, is more than two times less than that of \(d\)-tubocurarine (table 1 and fig. 3). The calculated autonomic margin of safety for metocurine is 50 to 50 times greater than that for \(d\)-tubocurarine in the cat (table 2).

**Discussion**

Known structure–activity relations among quaternary ammonium compounds support the development of increased neuromuscular blocking activity and lessened autonomic effects in bulky molecules when the number of quaternary functions is increased from one to two, albeit especially when the distance separating the quaternary nitrogen atoms is greater than 10 angstroms. This has been shown to be true of bisbenzylisouquinoline alkaloids (e.g., \(d\)-tubocurarine, a monoquaternary, and metocurine, a diquaternary), as well as mono- and diquaternary steroidal molecules. Both Durant, Bowman and Marshall\(^{13}\) and Hughes and Chapple\(^{7,8}\) describe the separation of the neuromuscular blocking action from the autonomic effects as far greater for metocurine than for \(d\)-tubocurarine. Their data appear to be roughly comparable to ours, and indicate that the increased separation is due mainly to the markedly (9.1 to 14 times) higher neuromuscular blocking potency of metocurine than \(d\)-tubocurarine in the cat. The modest quantitative differences between our study and the results of those of Durant et al.\(^{11}\) and Hughes and Chapple\(^{7}\) are probably due to slight variations in methodology, namely in the durations and frequencies of autonomic stimulation and in our use of cumulative dose–response curves and the neuromuscular ED\(_{90}\) rather than ED\(_{50}\) to calculate the dose ratios in table 2.

Our findings are also in agreement with the work of the above-mentioned investigators\(^{7,8,13}\) in locating the site of vagal block by \(d\)-tubocurarine at parasympathetic ganglia. All three investigations indicated that the vagolytic action of metocurine is one to three times weaker than that of \(d\)-tubocurarine in the cat. Durant et al.\(^{11}\) concluded that metocurine blocks muscarinic receptors. The present study also demonstrated muscarinic receptor blockade by metocurine, but the

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**Table 1. Relative Properties of Metocurine and \(d\)-Tubocurarine in the Cat**

<table>
<thead>
<tr>
<th>Action</th>
<th>Metocurine (ED(_{50}), mg/kg)</th>
<th>(d)-Tubocurarine (ED(_{50}), mg/kg)</th>
<th>Potency Ratio (ED(<em>{50}), Metocurine/ED(</em>{50}), (d)-Tubocurarine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuromuscular block</td>
<td>0.025* (0.007–0.089)</td>
<td>0.35 (0.12–1.02)</td>
<td>14.0</td>
</tr>
<tr>
<td>Sympathetic block</td>
<td>4.40* (2.05–9.46)</td>
<td>1.35 (0.59–3.11)</td>
<td>0.31</td>
</tr>
<tr>
<td>Vagal block</td>
<td>0.85* (0.33–1.36)</td>
<td>0.29 (0.15–0.58)</td>
<td>0.34</td>
</tr>
<tr>
<td>Histamine release</td>
<td>0.88* (0.48–1.60)</td>
<td>0.40 (0.22–0.72)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

* Significantly greater (or less) than the corresponding value for \(d\)-tubocurarine.

Dose–response data yielding ED\(_{50}\) for neuromuscular block and ED\(_{50}\) for vagal and sympathetic block were obtained in cumulative fashion. Data giving ED\(_{50}\) for histamine release represent bolus dosages. Parentheses include 95 per cent confidence limits.
latter effect appeared to occur at somewhat higher dosage than that necessary to block neurally-elicted bradycardia, suggesting a combined mechanism of vagal inhibition by metocurine, with the ganglionic effect predominating. Consistent with our data, Lee Son and Waud have listed the ratios $K_Kinatrium/K_{Inatrium}$ in the guinea pig for $d$-tubocurarine and metocurine as 264 and 136, respectively. These figures indicate that, relative to its neuromuscular blocking action, the ability of metocurine to block muscarinic receptors is nearly twice that of $d$-tubocurarine. Many potent quaternary neuromuscular blocking drugs do block cardiac muscarinic receptors, so such an action of metocurine is not unexpected. The dosage separation of the latter effect from its neuromuscular blocking action is very wide, however; consequently, the antimuscarinic action is not apparent clinically.

We agree with Durant et al. and Hughes and Chapelle that sympathetic block occurs at the ganglia with both $d$-tubocurarine and metocurine, the latter being 3.1 (present study) to 6.7 (Durant et al.) times less potent in this respect.

We have used the ED$_{30}$ for neuromuscular blockade to calculate the dose ratios indicating the autonomic margins of safety because this level of neuromuscular block is clinically pertinent, nearly always corresponding to good abdominal relaxation and usually compatible with sufficient jaw and laryngeal relaxation to allow reasonably smooth tracheal intubation. Fifty per cent block is usually not compatible with good clonical relaxation.

No previous work has quantitatively compared the dose-response curves for histamine release by metocurine and by $d$-tubocurarine. McCullough et al. did not find any histamine release in the cat after metocurine, 0.4 mg/kg, a dose about ten times greater in terms of neuromuscular blocking potency than the same dose of $d$-tubocurarine, which significantly increased plasma histamine levels. The present study showed that the occurrence of histamine release as indicated by the delayed depressor response is dose-related. The latter response is clinically important, since it represents a quantity of histamine release that produces an easily observable and clinically significant cardiovascular effect. It has been used as an assay for histamine release in both the dog and the cat.

Dose ratios for autonomic vs. neuromuscular blocking actions of relaxants (autonomic margins of safety) probably constitute more appropriate therapeutic indices for these drugs than the usually determined LD$_{30}$/ED$_{30}$ ratio, since the relaxants are commonly given only to the artificially ventilated animal or patient. Under such circumstances neuromuscular blocking agents cause death secondary to cardiovascular effects mediated through the autonomic nervous system, rather than to paralysis of skeletal muscle. An index that quantitates the dose interval separating the autonomic effects of neuromuscular blocking drugs from their actions on skeletal muscle in man during anesthesia should be helpful in terms of understanding the total pharmacologic spectrum of each relaxant. Such an index might facilitate the choice of a specific relaxant having the particular cardiovascular effect best suited to the individual patient and the planned anesthetic and surgical procedures.

Calculation of autonomic margins of safety for neuromuscular blocking drugs in man is not possible because suitable methods for quantitating autonomic responses in man are not available. There is considerable indication, however, that when a neuromuscular blocking agent produces an autonomic effect in the cat within or near the neuromuscular blocking dose range, then neuromuscular blockade in man will be accompanied by cardiovascular changes corresponding to those autonomic actions. Thus, for example, tachycardia always occurs together with neuromuscular blockade by gallamine in man, consistent with the fact that gallamine’s dose-response curves for neuromuscular and vagal block in the cat are superimposed. Pancuronium, on the other hand, generally causes less increase in heart rate in man than gallamine. This correlates well with observations in cats that the dose-response curve for vagal block by pancuronium lies to the right of the curve for neuromuscular block; only in the dose range producing high-grade neuromuscular inhibition does modest vagolysis occur. Similarly, the comparatively weaker hypotensive action of metocurine vs. $d$-tubocurarine in man corresponds well to metocurine’s lesser ganglionic-blocking and histamine-releasing potencies in the cat, as described in the present work, as well as in other studies.

Autonomic margins of safety calculated for experimental neuromuscular blocking agents in the cat might be useful in anticipating the qualities and magnitudes

| Table 2. Autonomic Margins of Safety of Metocurine and $d$-Tubocurarine in the Cat |
|-----------------|-----------------|-----------------|
| Dose Ratio*    | Metocurine      | $d$-Tubocurarine |
| ED$_{10}$        | 176.0           | 3.86            |
| ED$_{30}$        | 34.0            | 0.83            |
| ED$_{30}$        | 35.2            | 1.14            |

* Dosages from table 1.

of autonomic effects the drugs might have in man. Thus, for example, the tachycardia produced by the new relaxant fazadinium (AH8165) during clinical use might have been expected in view of data from cats showing that the dose–response curve for vagal blockade lies to the left of the curve for neuromuscular blockade.

High doses of AH8165 produce a decrease in peripheral resistance and hypotension in man. Such effects might also have been predicted from experiments on the cat that demonstrated a ganglion-blocking action whose dose–response curve is located just to the right of that for neuromuscular blockade. The cardiovascular effects under clinical conditions of another new relaxant, BWY100 (AA-136), were anticipated from autonomic studies done in cats, where a muscarinic vagolytic property was found to overlie the dose–response curve for neuromuscular block, and a ganglion-blocking action occurred at a dosage greater than that necessary for 100 per cent twitch inhibition. Thus, tachycardia was seen at all dosage levels in man, and hypotension developed only secondary to dosages producing more than 95 per cent twitch depression.

References

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