Title: REVERSAL OF THE D-TUBOCURARINE BLOCK BY COMBINATIONS OF METHYLGUANIDINE AND CHOLINESTERASE INHIBITORS IN VITRO

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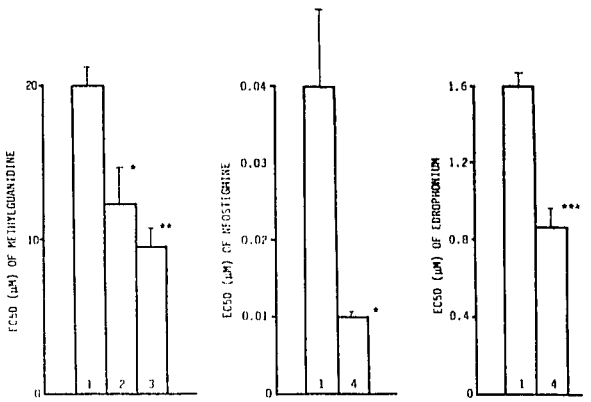
Introduction. Methylguanidine (MeG) inhibits the development and antagonizes the neuromuscular (NM) blocking effect of nondepolarizing muscle relaxants (1). The antagonist effect of MeG, similarly to that of 4-aminopyridine (4AP), is due to the facilitation of the evoked release of acetylcholine (ACh) from the motor nerve terminal (2). It has been reported that in rats the antagonist effect of combinations of 4AP and cholinesterase inhibitors (anti-ChE) is more than additive (3,4). In the present study the antagonism of d-tubocurarine (d-Tc) induced NM block by combinations of MeG with neostigmine (NEO) or edrophonium (EDR) has been investigated.

Methods. Male Sprague-Dawley rats weighing 275 to 375 g were lightly anesthetized with ether and decapitated. Their phrenic nerve-hemidiaphragm preparations were suspended in modified Krebs' solution having the same $[Ca^{++}]$ and $[Mg^{++}]$ as rat plasma. Thebath was aerated with 95% O_2-5% CO_2 and its temperature was kept at 37°C; pH 7.38 to 7.42. The phrenic nerves were stimulated with supramaximal, square wave impulses of 0.2 ms duration at 0.1 Hz. The isometric force of contraction (P) of the muscles was quantitated by FT03 transducers and continuously recorded. After P became stable an about 90% neuromuscular (NM) block was produced by adding the appropriate concentration of d-Tc. Subsequently the concentrations of MeG, NEO or EDR that increased P from < 10% to 50% of control (EC50) was determined from the computer derived, cumulative, log dose-effect regression lines. In other experiments the EC50 of MeG was determined after the preliminary addition of 0.01 μM NEO or 0.3 μM EDR. Similarly the EC50 of NEO and EDR was determined after the addition of 5 μM MeG. Data were analyzed with ANOVA followed by Tuckey's (5) test or Student's t test; $p < 0.05$ was considered significant.

Results. Data presented in fig. 1 demonstrate that the preliminary addition of relative low concentrations of NEO or EDR significantly decreased the EC50 of MeG. Similarly the preliminary addition of MeG significantly decreased the EC50 of NEO or EDR. The isobolograms constructed from the antagonism data (fig. 2) indicate that, whatever the sequence of addition of MeG, NEO or EDR to the bath their combined antagonist effect is more than additive (synergistic).

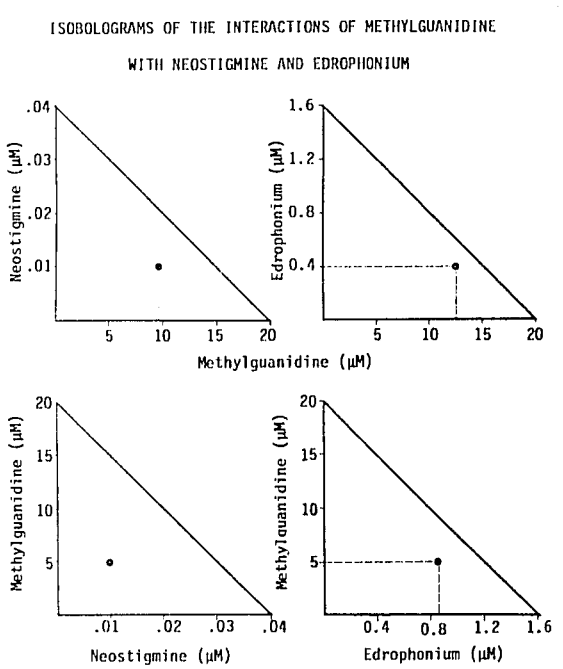
Discussion. The findings indicate that the profound steady state d-Tc block can be antagonized by the combination of relatively low concentrations of MeG, a compound that increases the evoked release of ACh (2) and NEO or EDR which inhibit enzymatic hydrolysis of ACh. MeG is an endogenous substance which is readily excreted through the kidneys. If the potentiation of the antagonist effect of anti-ChE by MeG can be substantiated in in vivo animal experiments, investigation of the antagonism of residual NM block at the end of anesthesia by combinations of small doses of MeG and anti-ChE may be warranted.

Fig. 1 EC50 Values



1. Compound alone; 2. MeGpreceeded by 0.01 μM NEO; 3. MeG preceeded by 0.3 μM EDR; 4. NEO or EDR preceeded bu 5 μM MeG

Fig 2



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