Atropine and neuromuscular fade in the mouse phrenic nerve-diaphragm

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Purpose: These studies were intended to resolve the conflict between the reasonable inference from the scientific literature that atropine might alter neuromuscular fade and the expectation from informal clinical experience that it does not.

Methods: We examined the effect of a high concentration of atropine (20 μ M) on moderate neuromuscular block and fade produced by d-tubocurarine (dTC). Isometric twitch tension was measured in the mouse phrenic nerve-diaphragm preparation. In one set of experiments, the phrenic nerve was stimulated with trains of 5 pulses at 10 Hz every second. Block and fade were measured in two groups, control and with atropine ($n = 6$ each). In another set of experiments, the phrenic nerve was stimulated with standard train-of-four stimulation (TOF, 4 pulses at 2 Hz every 11.5 seconds). Block and fade were measured first in a control period and then in a treatment period with either saline ($n = 4$) or atropine ($n = 4$).

Results: During 10 Hz train stimulation, atropine had no significant effect on either the block of the first twitch (control: 62 ± 17 ; atropine: 75 ± 4) or fade (control: 55 ± 12 ; atropine: 57 ± 14) produced by dTC. Similarly, atropine did not differ significantly from saline in altering dTC-induced block of first twitch (saline: 92.5 ± 14 ; atropine $92.5 \pm 9.6\%$ control) or fade (saline 119 ± 50 ; atropine $102 \pm 30\%$ control) during TOF stimulation.

Key words

ANTIMUSCARINIC DRUGS: atropine;
MONITORING: Neuromuscular block, fade, train-of-four;
NEUROMUSCULAR RELAXANT: d-tubocurarine;
RECEPTORS: acetylcholine.

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Conclusions: While atropine may alter ACh release under some conditions, its action is not great enough to alter either block or fade.

Objectif: Résoudre la controverse entourant la déduction logique basée sur les publications selon laquelle l'atropine pourrait altérer le fade et notre croyance fondée sur l'expérience clinique informelle qu'elle ne l'altère pas.

Méthodes: Les auteurs ont étudié les effets d'une concentration élevée d'atropine (20 μ M) sur un block neuromusculaire modéré et le fade produits par la d-tubocurarine (dTC). La tension isométrique du twitch a été mesurée sur une préparation nerf phrénique-diaphragme. Au cours d'une série d'expériences, le nerf phrénique a été stimulé avec des ondes de cinq pulsations à 10 Hz à la seconde. Le bloc et le fade ont été mesurés dans deux groupes, contrôle et atropine ($n = 6$ chacun). Dans une autre série d'expériences, le nerf phrénique a été stimulé au train-of-four standard (TOF, 4 stimuli à 2 Hz à toutes les 11,5 sec). Le bloc et le fade ont été d'abord mesurés pendant la période de contrôle, ensuite avec du sol. physiologique (SP) ($n = 4$) ou de l'atropine ($n = 4$).

Résultats: Pendant la stimulation de 5 pulsations à 10 Hz, l'atropine n'a pas eu d'effets significatifs sur le bloc du premier twitch (contrôle: 62 ± 17 ; atropine: 75 ± 4) ou le fade (contrôle: 65 ± 12 ; atropine: 57 ± 14) produit par la dTC. De la même façon, l'atropine n'a pas été différente du SP en ce qui concerne l'altération du bloc du premier twitch induit par la dTC (SP: $92,6 \pm 14$; atropine $92,5 \pm 9,6\%$ du contrôle) ou du fade (SP: 118 ± 50 ; atropine $102 \pm 30\%$ du contrôle) pendant le TOF.

Conclusion: Bien que l'atropine puisse altérer la libération d'ACh sous certaines conditions, son action n'est pas assez puissante pour altérer ni le bloc ni le fade.

There is strong evidence that muscarinic drugs alter acetylcholine (ACh) release at the neuromuscular junction. Direct evidence comes from experiments in which the release of radiolabeled ACh was measured. Atropine greatly increased evoked ACh release from short trains (40 pulses) of stimulation at 5 and 50 Hz,¹ while scopolamine increased release from shorter trains (100 pulses)

but decreased the release from longer trains (1500 pulses) at either 5 or 25 Hz.² Strong indirect support for the finding that muscarinic antagonists increase ACh release comes from experiments in which atropine increased total Ca^{++} currents at rat motor nerve endings stimulated at 2 Hz and blocked by d-tubocurarine (dTC).³

These recent findings demonstrating prejunctional actions of muscarinic drugs raise the issue of whether muscarinic antagonists affect neuromuscular fade. The neuromuscular fade observed in the presence of non-depolarizing blocking drugs might well be altered by the prejunctional actions of muscarinic antagonists since fade is a reflection of decreases in ACh release.⁴ Additionally, it is important to know if muscarinic antagonists affect train-of-four stimulation since both are used widely in the practice of anaesthesia. Thus, in the absence of any published studies directly examining whether muscarinic drugs affect neuromuscular fade, we tested whether atropine has an effect on moderate levels of block and fade produced by dTC.

Methods

Following approval of the institutional animal care and use committee, Male NIH Swiss mice (29–35 g) were killed by cervical dislocation. The diaphragm and part of its phrenic nerve were dissected out and prepared for the measurement of twitch tension as in a modified Krebs medium at 37°C as previously described,⁵ except that the whole left hemidiaphragm was used instead of a strip. Initially (8 of 12 experiments), we conducted pilot experiments in anticipation that we might wish to measure the effects of atropine on ACh release, which required retrograde vascular perfusion ($65\text{--}75\ \mu\text{l} \cdot \text{min}^{-1}$) via the diaphragmatic vein. However, as it became clear that atropine was not having substantial effects on neuromuscular transmission, we conducted the rest of the experiments using a traditional 50 ml organ bath.

Resting length was adjusted to maximize twitch tension at 0.2 Hz. The phrenic nerve was stimulated with trains of five pulses at 10 Hz every second. At this stimulation frequency in the mouse, summation does not occur and the individual twitch heights within the train can be determined. Neuromuscular block was calculated as the percent decrease in the tension of the first twitch of the train (T_1) from the pre-dTC 0.2 Hz control. Neuromuscular fade was calculated as the percent decrease in tension of the fifth to the first twitch in the train-of-five, i.e., $(1 - T_5/T_1) \times 100$, where T_5 was the fifth twitch in the train. Block and fade were measured in the presence of $1.0\ \mu\text{M}$ dTC either alone (saline group, $n = 6$) or with $20\ \mu\text{M}$ atropine (atropine group, $n = 6$).

We re-examined the effect of atropine on neuromus-

FIGURE The percent of block and fade during trains of five pulses at 10 Hz in the presence of $1.0\ \mu\text{M}$ dTC does not differ between saline (i.e., control) and atropine ($20\ \mu\text{M}$) groups.

cular block and fade using a slightly different protocol with a lower dose of dTC ($0.75\ \mu\text{M}$, in an attempt to bring block closer to 50%) and train-of-four stimulation (four pulses at 2 Hz every 11.5 seconds, to have greater clinical relevance). Phrenic nerve diaphragms were prepared for a traditional organ bath as described above. In a control period, all the preparations were incubated with dTC for 15 min at which time block and fade were determined in the standard manner.⁵ Then, preparations were treated for 15 min with fresh dTC to which either saline ($n = 4$) or atropine ($20\ \mu\text{M}$, $n = 4$) was added and block and fade were determined again.

Data are presented as mean $\pm$ SEM. Groups were compared by *t* tests and $P < 0.05$ was considered significant.

Results

There were no differences between the saline and atropine groups in either neuromuscular block or fade during stimulation with trains of five pulses at 10 Hz every second in the presence of $1.0\ \mu\text{M}$ dTC (Figure).

Similarly for train-of-four stimulation, there were no differences between the saline and atropine groups in percent change from the control period of either block (saline: $92.5 \pm 14.8\%$; atropine: $92.5 \pm 9.6\%$) or fade (saline: $119 \pm 49.8\%$; atropine: $102 \pm 30\%$). There were no differences between the groups after the addition of saline or atropine for either block (saline: 43 ± 3.0 ; atropine: 37 ± 4.6) or fade (saline: 18 ± 2.3 ; atropine: 23 ± 9.6). Thus, atropine affected neither block nor fade during train-of-four stimulation in the presence of dTC.

Discussion

The common clinical impression is that atropine does not alter the response to train-of-four stimulation in the presence of non-depolarizing blocking drugs. In the absence of a formal clinical study, the present results provide the strongest support for this impression by showing that even a high concentration of atropine affected neither neuromuscular block nor fade in partially paralyzed *in vitro* mouse tissues.

We are aware of work by Alves-do-Prado *et al.*⁶ which shows that the time for recovery of tetanic fade in cats is accelerated by atropine. Since pharmacokinetic factors can alter train-of-four fade,⁵ we suspect that the effect of atropine found by Alves-do-Prado *et al.*⁶ is peculiar to the *ia* administration of dTC used in their cat model. However, it may be that atropine can alter fade during long trains of tetanic stimulation.

The present results also need to be seen in the context of other experiments also performed in rodents¹⁻³ which show that muscarinic receptor antagonism can alter ACh release from motor nerves. We do not contest these findings. However, these findings were made under conditions in which twitch tension could not be measured and thus did not indicate the effect of muscarinic antagonism on overall transmission (i.e., twitch tension). We conclude that whatever the effects of atropine on ACh release, they are too small to alter substantially the block or fade seen with short trains of sub-tetanic stimulation during partial paralysis.

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