Effects of Neuromuscular Presynaptic Muscarinic M₁ Receptor Blockade on Rocuronium-induced Neuromuscular Blockade in Immobilized Tibialis Anterior Muscles

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Backgrounds

➤Several studies using varied models of immobilization have shown that disuse of muscle leads to muscle atrophy, and *de novo* expression of immature AChRs throughout the muscle membrane, despite the presence of continued innervations.

➤Neuronal nicotinic AChRs containing five homometric $\alpha 7$ -subunits, previously described only in the central nervous system, have more recently been described in skeletal muscle after denervation only.

➤Immobilization-induced muscle atrophy leads to de novo junctional-area expression of $\alpha 7$ AChRs, which play a small but significant role in neurotransmission

➤Muscarinic facilitatory M₁ and inhibitory M₂ receptors regulate the fine-tuning actions of release of acetylcholine on neuronal firing, especially M₁ receptors have a predominant role at low frequency (<5Hz) stimulation.

➤In this study using mechanomyographic techniques together with pirenzepine in wildtype and $\alpha 7$ knockout ($\alpha 7$ KO) mice, we tested the hypothesis that acetylcholine (ACh) release modulation by muscarinic receptors is changed in neuromuscular junction of the muscles after immobilization and thus the tensions of the muscles during neuromuscular monitoring of rocuronium-induced neuromuscular block are changed.

Methods

➤The study protocol was approved beforehand by the Ethics Committee of the Laboratory of Animal Research, Asan Institute of Life Science (Seoul, Republic of Korea) on February 11, 2016 (Protocol No. 2016-13-023).

➤A total of 20 C57BL/6 (wildtype) and 10 $\alpha 7$ knock out ($\alpha 7$ KO) mice were used in this experiment. All animals were raised at a constant temperature of 22°C and maintained under a regular diurnal cycle with food and water supplied *ad libitum*.

➤The pinning-immobilization model was used for the current studies.

Conclusions

➤In wildtype mice, resistance to NMBA at immobilize side was diminished when the muscarinic M₁ receptor was blocked by specific antagonist, pirenzepine.

➤In $\alpha 7$ KO mice, resistance to NMBA at immobilize side was less than wildtype mice, and there were no changes in differences between control and immobilized side when the muscarinic M₁ receptor was blocked by specific antagonist, pirenzepine.

➤Expression of $\alpha 7$ AChR due to the disuse atrophy may have different response to the blockade of presynaptic M₁ receptor which made diminished release of ACh on neuronal stimulation.

References

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Results

Table 1. Comparison of wehole body weight at initial and 2wks after immobilization , tibialis anterior musle weight of immobilize side and contralateral side in wildtype and $\alpha 7$ KO mice.

	BW initial (g)	BW immo (g)	TA control (mg)	TA immo (mg)
Wild type	27.32 ± 1.87	25.51 ± 1.63*	46.09 ± 3.82	35.77 ± 4.19*
$\alpha 7$ KO	27.21 ± 1.55	26.14 ± 1.49*	46.99 ± 3.11	34.79 ± 5.80*

Data are expressed as mean ± SD. $\alpha 7$ KO: $\alpha 7$ knock out mice. BW initial: body weight at initial. BW immo: body weight of 2wks after immobilization. TA control: tibialis anterior muscle weight of contralateral side. TA immo: tibialis anterior muscle weight of immobilize side. (* p<0.05 comparing with the other in the same genotype)

Table2. Comparison of dose responses of rocuronium between control and immobilize side at initial, PZP1, and PZP2

Wildtype mice					α7 KO mice				
		Control	immobilize	p value			Control	immobilize	p value
Initial	ED ₅₀	1.234 ± 0.248	2.395 ± 0.482	0.018	Initial	ED ₅₀	0.972 ± 0.230	1.521 ± 0.485	0.014
	ED ₉₅	1.790 ± 0.460	3.669 ± 0.677	0.018		ED ₉₅	1.318 ± 0.327	2.154 ± 0.799	0.014
PZP1	ED ₅₀	1.148 ± 0.422	2.239 ± 0.833	0.078	PZP1	ED ₅₀	0.909 ± 0.187	1.612 ± 0.608	0.014
	ED ₉₅	1.608 ± 0.600	3.298 ± 1.397	0.099		ED ₉₅	1.259 ± 0.258	2.289 ± 0.922	0.024
PZP2	ED ₅₀	1.497 ± 0.553	2.925 ± 1.475	0.099	PZP2	ED ₅₀	0.831 ± 0.095	1.504 ± 0.404	0.022
	ED ₉₅	2.065 ± 0.816	4.053 ± 2.043	0.126		ED ₉₅	1.153 ± 0.137	2.158 ± 0.816	0.028

Fig.1 Comparison of the progression of the T1 depression between immobilized and contrlateral normal side by the genotype. We set the regression equation which has the R² more than 0.8 and compared the values (Ω) which are represent their slope. There were statistically significant differeces in the Ω s in the $\alpha 7$ KO groups throughout the whole period, but those of wildtype group had statistical significance at only the initial period. Initial: reference T1 depression. PZP1: period at the injection of pirenzepine 0.2ug. PZP2: period at the injection of pirenzepine 2 ug

Table 3. Comparison by the genotype in dose response of rocuronium in the same side.

				Control side				Immobilize side			
				Wild	$\alpha 7$ KO	p value		Wild	$\alpha 7$ KO	p value	
initial	ED ₅₀			1.23 ± 0.25	0.97 ± 0.23	>0.05		2.40 ± 0.482	1.52 ± 0.49	0.02	
	ED ₉₅			1.79 ± 0.46	1.32 ± 0.33	>0.05		3.67 ± 0.677	2.16 ± 0.80	0.02	
PZP1	ED ₅₀			1.23 ± 0.25	0.91 ± 0.19	>0.05		2.24 ± 0.833	1.61 ± 0.61	>0.05	
	ED ₉₅			1.79 ± 0.46	1.26 ± 0.26	>0.05		3.30 ± 1.397	2.29 ± 0.92	>0.05	
PZP2	ED ₅₀			1.50 ± 0.55	0.83 ± 0.10	0.04		2.93 ± 1.475	1.50 ± 0.40	>0.05	
	ED ₉₅			2.07 ± 0.82	1.15 ± 0.14	0.04		4.05 ± 2.043	2.16 ± 0.65	>0.05