

## Streptozotocin Diabetes Attenuates the Effects of Nondepolarizing Neuromuscular Relaxants on Rat Muscles

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The hypothesis of this study was that diabetes-induced desensitization of rat *soleus* (SOL) and *extensor digitorum longus* (EDL) to non-depolarizing muscle relaxants (NDMRs) depends on the stage of diabetes and on the kind of NDMRs. We tested the different magnitude of resistance to vecuronium, cisatracurium, and rocuronium at different stages of streptozotocin (STZ)-induced diabetes by the EDL sciatic nerve-muscle preparations, and the SOL sciatic nerve-muscle preparations from rats after 4 and 16 weeks of STZ treatment. The concentration-twitch tension curves were significantly shifted from those of the control group to the right in the diabetic groups. Concentration giving 50% of maximal inhibition (IC<sub>50</sub>) was larger in the diabetic groups for all the NDMRs. For rocuronium and cisatracurium in both SOL and EDL, IC<sub>50</sub> was significantly larger in diabetic 16 weeks group than those in the diabetic 4 weeks group. For SOL/EDL, the IC<sub>50</sub> ratios were significantly largest in the diabetic 16 weeks group, second largest in the diabetic 4 weeks group, and smallest for the control group. Diabetes-induced desensitization to NDMRs depended on the stage of diabetes and on the different kind of muscles observed while was independent on different kind of NDMRs. The resistance to NDMRs was stronger in the later stage of diabetes (16 *versus* 4 weeks after STZ treatment). Additionally, when monitoring in SOL, diabetes attenuated the actions of neuromuscular blockade more intensely than that in EDL. Nonetheless, the hyposensitivity to NDMRs in diabetes was not relevant for the kind of NDMRs.

**Key Words:** Diabetes, *Extensor digitorum longus*, Non-depolarizing muscle relaxants, *Soleus*, Streptozotocin

### INTRODUCTION

Diabetes mellitus is characterized by peripheral neuropathy of sensory and motor nerves. Peripheral nerves and muscles dysfunctions have been demonstrated in both humans and rodents [1-3]. The morphological destabilization of neuromuscular junction (NMJ) in diabetes such as axonal degeneration, axonal atrophy and demyelination was has been verified [4,5].


It has been reported that the hyposensitivity to *d*-Tubocurarine and other non-depolarizing muscle relaxants (NDMRs) in diabetic state [6,7]. However, the hypersensitivity to acetylcholine (ACh) and succinylcholine due to the decrease of ACh release and the augmentation of

presynaptic acetylcholine receptors (AChRs) sensitivity was has been investigated [8,9]. The profound response to muscle relaxants in diabetes needs further investigation.

Mammalian skeletal muscle fibers are commonly divided into three types: slow-oxidative (type I), fast-glycolytic (type IIb), and fast-oxidative-glycolytic (type IIa). The typing of muscle fibers is based upon oxidative and glycolytic capacities, twitch characteristics, and ATPase activity [10]. *Extensor digitorum longus* muscle (EDL), and *soleus* muscle (SOL) are typical fast and slow twitch muscles, composed of predominately fast and slow twitch fibers respectively. Many previous studies have focused on the effects of diabetes on the muscle contractile properties, which depends on the alteration of muscle types [11]. The fiber-type switching from fast to slow isoforms was manifested in diabetes rodents [12]. Regarding the contractile properties, an increase in the contraction and relaxation times in SOL in part reflecting a shift in the isomyosin composition with diabetes, which was indicated by an increased percentage of histochemically demonstrable slow fibers, at the expense of fast fibers, was testified. Moreover, a decrease in tetanic

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**ABBREVIATIONS:** NMJ, neuromuscular junction; NDMRs, non-depolarizing muscle relaxants; ACh, acetylcholine; AChRs, acetylcholine receptors; EDL, *Extensor digitorum longus* muscle; SOL, *soleus* muscle; STZ, streptozotocin; IC<sub>50</sub>, concentration giving 50% of maximal inhibition.

tension in EDL with no effect on muscle strength and performance, which was relative to atrophy of the fast-glycolytic fibers normally making the largest contribution to tension, was proved [13].

Findings of previous studies have indicated the differences in muscle relaxation in slow and fast twitch muscles. It has been reported that type I muscle fiber is more resistant to NDMRs [14-17]. Motor neurons distributed to each muscle have muscle-specific characteristics that are reflected in differences in ACh release [18]. The switching of muscle fiber types affects motor neurons and NMJs subsequently. NMJs at type I and IIa fibers are smaller and less complex compared to NMJs at type IIx and/or IIb fibers [19]. The safety factor for neuromuscular transmission is higher in fast- than in slow-twitch muscles. In comparison with NMJs in slow-twitch muscles, those in fast muscles are reported to have a higher quantal content, more functional AChRs, a higher density of voltage-gated sodium channels in both junctional and extrajunctional regions. Postsynaptic folds are shorter, broader and sparser in slow-twitch muscles than in fast-twitch muscles [20]. The fiber-type switching results in different performances of SOL and EDL in diabetes, which may underpin the mechanisms of different effects to NDMRs in these two muscles.

Whilst the contractile and histochemical properties of SOL and EDL have been studied extensively, little is known about the pharmacodynamics of muscle relaxants in those muscles. Of note the fact is that NMJs are the very early target in diabetes, the effects of diabetes on NDMRs actions in SOL and EDL may be changed [5]. It is worth notice noticing that the progress in muscle functions and fiber alteration occurs in a time-dependent manner [4]. It has not been investigated whether the effects of diabetes on the actions of NDMRs depends on the stage of this disease or not has not been investigated.

The aim of this study was to test the differences in the magnitude of resistance to vecuronium, cisatracurium, and rocuronium in SOL and EDL at different stages of streptozotocin (STZ)-induced diabetes. The hypothesis of this study was that diabetes-induced desensitization to NDMRs depends on the stage of diabetes, different muscles observed, and on the kind of NDMRs. We investigated the actions of vecuronium, cisatracurium, and rocuronium on twitch tensions of the EDL sciatic nerve-muscle preparations, and the SOL sciatic nerve-muscle preparations from rats after 4 weeks and 16 weeks of STZ treatment.

## METHODS

### *Animals*

The study was approved by the Animal Care Committee in Shanghai Jiaotong University (Shanghai, China). Forty male Sprague-Dawley rats (Experimental Animal Center of the School of Medicine, Shanghai Jiaotong University, Shanghai, China), weighing 200 to 240 g, were housed in groups of three. They were fasted but allowed to have free access to water and food prior to the experiments.

### *Induction of diabetes*

Rats were randomly divided into two groups. One group (n=20) was made diabetic group by a single injection of STZ 60 mg/kg intraperitoneally after 12 hours fasting. Another

group (n=20) was given the same volumes of citric acid buffer solution, which was made up of normal animals matched in age. 2 days after injection, blood sugar was measured twice in series by cutting tails. Successful models were determined if the blood sugar values were above 16.7 mmol/L. The rats with blood sugar tested below 16.7 mmol/L were rejected from the study. After modeling successfully, the diabetic rats and the age-matched controls were randomly divided into two subgroups, diabetic 4 weeks group (dia 4 wk) and diabetic 16 weeks (dia 16 wk) group, control 4 weeks group (con 4 wk) and control 16 weeks group (con 16 wk) (n=10 each group) respectively. The rats were sacrificed at 4 and 16 weeks after STZ administration.

### *Nerve-muscle preparations*

12 rats were killed with 60 mg/kg pentobarbital intraperitoneally. The isolated EDL sciatic nerve-muscle preparations and the SOL sciatic nerve-muscle preparations were established for indirectly electrical stimulation as described previously [21,22]. Body temperature was maintained at 37°C using a heating blanket and radiant heat.

Either EDL or SOL was exposed in one leg. After measurements were completed for one muscle, the other was then exposed in the other leg. The SOL was exposed by sectioning the tendons connecting the plantaris and gastrocnemius muscles to the heel, and reflecting the muscles back. Silk thread was attached to the distal tendon of the SOL and the tendon was sectioned. The muscle was then carefully freed of surrounding tissues, ensuring the blood supply remained intact, and the sciatic nerve was sectioned. The EDL was prepared in the similar manner after first exposing the muscle by reflection of the anterior tibialis muscle. The silk sutures were tied to the proximal and distal tendons of the EDL and/or soleus muscles and the muscles were removed, tendon to tendon.

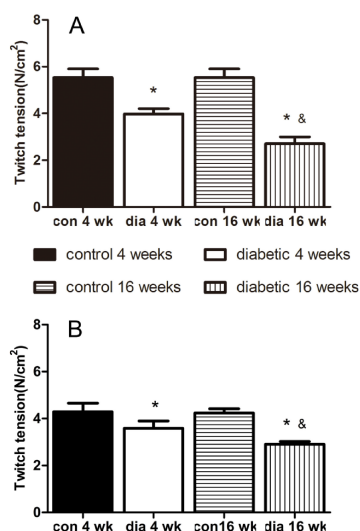
The isolated nerve-muscle preparations were dipped immediately into plexiglass chambers filled with Krebs solution, maintained at 37°C and bubbled with 95% oxygen/5% CO<sub>2</sub>. The composition of the Krebs solution was as follows: 137 mM NaCl, 4 mM KCl, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 1 mM KH<sub>2</sub>PO<sub>4</sub>, 12 mM NaHCO<sub>3</sub> and 6.5 mM glucose, with a pH 7.40±0.05 during bubbling.

### *NDMRs potency*

The indirect electrical stimulation-evoked twitch tension was recorded with MPA Multiple Channel Biological Signal Analysis System (provided by the Department of Anesthesiology, Shanghai First People's Hospital of SJTU). Each isolated strip was mounted vertically in a tissue chamber, inferiorly positioned. The EDL and SOL preparations were aligned vertically with distal tendon attached to the force displacement transducer (ALCM System for Isolated Tissue Organ Research; 40 ml in volume), proximal tendon fixed to the stainless steel fixed-post. The chamber was filled with Krebs solution as mentioned above. The nerves of the preparations were positioned on wire bipolar platinum electrodes for indirect stimulation. Isometric tension was elicited by indirect supramaximal constant voltage stimulation at 0.1 Hz for 0.05 ms, using a stimulator and a constant voltage unit. The twitch tension was recorded via the force transducer on a recorder (ALCMPA 2000m, Acquisition and Analysis System for Life Science Research, Shanghai Alcott Biotech, Shanghai, China). The stimulator

was activated by a personal computer. Twitch stimuli were used to determine the optimal length (L0) at which skeletal muscle may generate the greatest force, followed by a 15 min thermo-equilibration period. A number of measurements were performed as described in subsequent sections.

After the elicited twitch tension had been stabilized for a minimum of 15 min, the singletwitch tension, averaged in groups of five, was determined. The proximal end of the nerve attached to the strips was stimulated with a single supramaximal train of rectangular pulses (stimulation intensity which is the minimal stimulation intensity that can induce maximal force of contraction, duration 0.2 ms). The pulse was repeated three times at 5-second intervals, and the mean muscular tension amplitude was calculated. Before vecuronium, cisatracurium or rocuronium was added in, the baseline twitch tension amplitude was recorded as the control value. The NDMR was then applied to the preparation at the concentration of 0.01, 0.1, 0.5, 1, 2, 5, 7, 10, 12, 15, 20, 30, 40, 50  $\mu\text{mol/l}$ . Drug concentrations were determined by adding freshly prepared solutions with calibrated micropipettes to modified Krebs solution (40 ml) in the tissue chamber. Following stabilization of the drug effect for a minimum of 10 min, singletwitch tension was again determined. Data were accepted when twitch tension returned to 95~105% of the initial value by rinsing the diaphragm preparation with Krebs solution in each study. Concentration-twitch tension curves were constructed and the values of concentration giving 50% of maximal inhibition ( $\text{IC}_{50}$ ) were obtained. Rocuronium bromide and vecuronium were obtained from N.V. Organon (Oss, The Netherlands), while cisatracurium from GlaxoSmithKline (Italy). And all other drugs were purchased from SigmaAldrich (St. Louis, MO, USA).



**Fig. 1.** Twitch tension height elicited by indirect stimulation in *Extensor digitorum longus* (EDL, A) and *Soleus* (SOL, B). There was a significant difference between diabetic groups and the age-matched control groups ( $p < 0.05$ ), and between dia 4 wk and dia 16 wk groups ( $p < 0.05$ ), while no significant difference between con 4 wk group and con 16 wk group. \* $p < 0.05$  versus control groups, & $p < 0.05$  versus diabetic 4 weeks group. con 4 wk, control 4 weeks group; dia 4 wk, diabetic 4 weeks group; con 16 wk, control 16 weeks group; dia 16 wk, diabetic 16 weeks group.

## Statistical analysis

Competition analysis data ( $\text{IC}_{50}$ ) and slope at  $\text{IC}_{50}$  were determined from a four-variable logistic sigmoidal dose-response model fitted to the concentration-twitch tension curves (all values were considered for analysis) with the computer program Prism 4 (GraphPad Software, Inc., San Diego, CA). All other data were analyzed by SPSS version 13.0 software (IBM, Armonk, NY). Data are expressed as mean $\pm$ SD. One- or two-way analysis of variance was used to test the significance of differences among all groups. The paired t test was used for comparison between any two groups.  $p < 0.05$  was considered to indicate a statistically significant difference.

## RESULTS

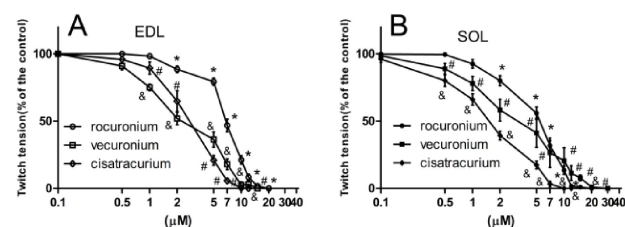
### Blood glucose levels

At the time of sacrifice, the level of glycemia was measured by glucose-strip reader and found to be  $23.11 \pm 5.05$  versus  $5.60 \pm 0.41$  mmol/l for dia 4 wk group and age-matched control animals, respectively ( $n=10$  for each experimental group,  $p < 0.01$ ), not increasing significantly with diabetes progression,  $26.21 \pm 5.61$  versus  $5.71 \pm 1.11$  mmol/l in dia 16 wk rats and age-matched controls, respectively ( $n=10$  for each group,  $p < 0.01$ ).

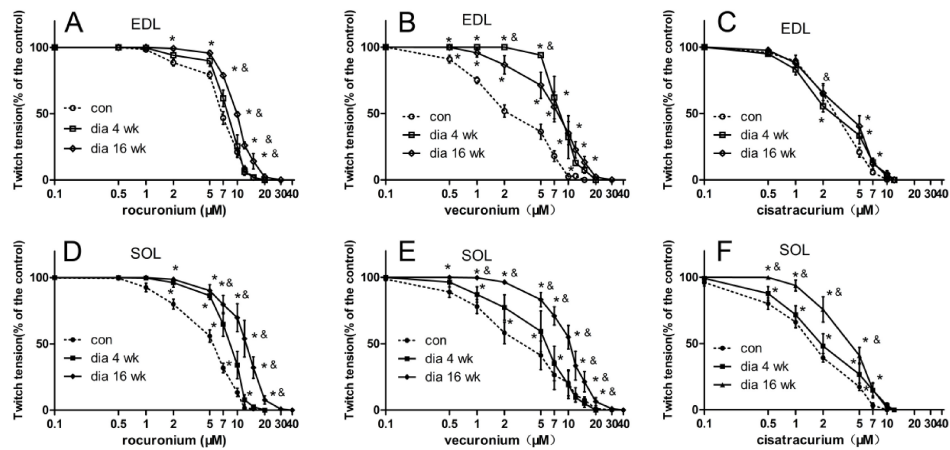
### NDMRs potency in nerve-muscle preparations

The nerve-muscle preparations ( $n=10$  each group) were established for indirectly electrical stimulation. After rinsing the muscle strips that had been exposed to NDMR with modified Krebs solutions, the indirectly elicited twitch tension returned to 95~105% of the initial value in each study. There were significant differences in the twitch tensions between diabetic groups and the age-matched control groups (Fig. 1;  $p < 0.05$ ), and between dia 4 wk and dia 16 wk groups (Fig. 1;  $p < 0.05$ ), while there was no significant difference between con 4 wk group and con 16 wk group (Fig. 1;  $p > 0.05$ ).

In all the groups, rocuronium, vecuronium and cisatracurium reduced the magnitudes of indirectly elicited



**Fig. 2.** Concentration-twitch tension curves for rocuronium, vecuronium, and cisatracurium in EDL (A) and SOL (B) in normal rats. The twitch tension was elicited by indirect stimulation at 0.1 Hz. The concentration-twitch tension curves were significantly different ( $p < 0.05$ ). Values are mean $\pm$ SD ( $n=10$  each). \* $p < 0.05$  versus control value of twitch tension for rocuronium, # $p < 0.05$  versus control value for vecuronium, and & $p < 0.05$  versus control value for cisatracurium. EDL, *Extensor digitorum longus*; SOL, *Soleus*.



**Fig. 3.** Concentration-twitch tension curves for rocuronium (A, D), vecuronium (B, E), and cisatracurium (C, F). The twitch tension was elicited by indirect stimulation at 0.1 Hz. Values are expressed as mean $\pm$ SD (n=10 each). The concentration-twitch tension curves of these groups were significantly different for each NDMRs compared with that of control value ( $p < 0.05$ , data not presented here). For rocuronium (A, D), vecuronium (B, E), and cisatracurium (C, F), there was a significantly difference between the curves in the dia 4 wk or dia 16 wk group and those in control group ( $p < 0.05$ ). For rocuronium (A, D), vecuronium (B, E), and cisatracurium (F), there was a significantly difference between the curves in the dia 4 wk group and those in dia 16 wk group ( $p < 0.05$ ). But there was not significantly difference between the curves in the dia 4 wk group and those in dia 16 wk group for cisatracurium in EDL ( $p > 0.05$ ). \* $p < 0.05$  versus con group, & $p < 0.05$  versus dia 4 wk group. EDL, *Extensor digitorum longus*; SOL, *Soleus*; con, control group; dia 4 wk, diabetic 4 weeks group; dia 16 wk, diabetic 16 weeks group.

**Table 1.** IC<sub>50</sub> values and slopes of the concentration-twitch tension curves of NDMRs

	Control		Diabetic 4 weeks		Diabetic 16 weeks	
	EDL	SOL	EDL	SOL	EDL	SOL
<b>Rocuronium</b>						
IC <sub>50</sub>	7.170 (4.705 ~ 8.667)	6.686 (5.148 ~ 8.684)	7.970 (7.361 ~ 8.667)	8.461 (7.590 ~ 9.432)*	9.832 (9.441 ~ 10.24)*&	12.84 (11.06 ~ 14.91)*&#
Slope	-3.727 $\pm$ 0.395	-1.667 $\pm$ 0.227 <sup>#</sup>	-4.737 $\pm$ 0.673	-4.030 $\pm$ 0.651*	-4.245 $\pm$ 0.306	-3.179 $\pm$ 0.630* <sup>#</sup>
<b>Vecuronium</b>						
IC <sub>50</sub>	4.798 (1.778 ~ 12.95) <sup>§</sup>	4.620 (1.354 ~ 15.77) <sup>§</sup>	8.163 (7.253 ~ 9.186)*	8.227 (4.215 ~ 17.37)*	8.532 (6.308 ~ 11.54)*	10.43 (8.801 ~ 12.37)*&# <sup>§</sup>
Slope	-0.932 $\pm$ 0.193 <sup>§</sup>	-0.930 $\pm$ 0.303 <sup>§</sup>	-2.336 $\pm$ 0.845* <sup>§</sup>	-1.278 $\pm$ 0.500* <sup>§</sup>	-1.973 $\pm$ 0.473* <sup>§</sup>	-2.639 $\pm$ 0.477* <sup>§</sup>
<b>Cisatracurium</b>						
IC <sub>50</sub>	2.962 (2.383 ~ 3.681) <sup>®</sup>	1.946 (1.361 ~ 2.782) <sup>®&amp;#</sup>	3.806 (2.177 ~ 6.652) <sup>®&amp;#</sup>	2.776 (1.098 ~ 7.020) <sup>®&amp;#</sup>	4.887 (2.713 ~ 8.802) <sup>®&amp;#</sup>	5.074 (3.213 ~ 8.010) <sup>®&amp;#</sup>
Slope	-1.980 $\pm$ 0.300 <sup>®</sup>	-1.221 $\pm$ 0.194 <sup>®</sup>	-1.224 $\pm$ 0.219 <sup>®</sup>	-1.080 $\pm$ 0.325 <sup>®</sup>	-1.334 $\pm$ 0.262 <sup>®</sup>	-1.689 $\pm$ 0.359 <sup>®</sup>

IC<sub>50</sub>, and slope at log IC<sub>50</sub> were determined from a four-variable logistic sigmoidal dose-response model fitted to the concentration-twitch tension curves. The twitch tension was elicited by indirect stimulation at 0.1 Hz. Values are expressed as means with 95% confidence intervals in IC<sub>50</sub> ( $\mu$ M) and mean $\pm$ SD in slope at log IC<sub>50</sub> (n=10 each). Statistical analysis was performed by oneANOVA with postBonferroni testing. \* $p < 0.05$  versus control group (Data of 16 weeks control group not presented in this table), & $p < 0.05$  versus diabetic 4 weeks group; # $p < 0.05$  versus EDL in the same group; § $p < 0.05$  versus Rocuronium, ® $p < 0.05$  versus Vecuronium. EDL, *Extensor digitorum longus*; SOL, *Soleus*.

twitch tensions dose-dependently (Fig. 2 and 3). For EDL and SOL in the control group, the concentration-twitch tension curves of rocuronium, vecuronium, and cisatracurium were significantly different ( $p < 0.05$ ; Fig. 2). Compared with vecuronium and cisatracurium, the curves were significantly shifted from rocuronium to the right in both EDL and SOL in the control group. For vecuronium in the control group, the curves were significantly shifted from cisatracurium to the right in SOL. IC<sub>50</sub>, was significantly largest for rocuronium, second largest for vecuronium, and smallest for cisatracurium in two muscles in the control

group ( $p < 0.05$ ; Table 1). The slope for rocuronium was significantly smaller than those for vecuronium and cisatracurium in the control rats ( $p < 0.05$ ; Table 1). For every NDMRs, IC<sub>50</sub> was significantly larger in EDL than in SOL in the control group. To be mentioned, the concentration-twitch tension curves and IC<sub>50</sub> of con 16 wk group were quite the same as those of con 4 wk group, so only the data of con 4 wk group was presented in this report.

For EDL and SOL, the concentration-twitch tension curves of rocuronium, vecuronium, and cisatracurium were significantly different in the diabetic groups compared with

**Table 2.** IC<sub>50</sub> ratios of the twitch tension depression by NDMRs in EDL and SOL

IC <sub>50</sub> ratios		Rocuronium	Vecuronium	Cisatracurium
dia 4 wk/con	EDL	1.112 (0.989~1.381)	1.701 (1.521~1.981) <sup>§</sup>	1.285 (1.014~1.561) <sup>#</sup>
	SOL	1.265 (1.011~1.589)	1.781 (1.421~1.931) <sup>§</sup>	1.427 (1.121~1.593) <sup>#</sup>
dia 16 wk/con	EDL	1.371 (1.015~1.621)	1.778 (1.510~1.981) <sup>§</sup>	1.650 (1.362~1.854) <sup>§</sup>
	SOL	1.870 (1.641~2.011) <sup>*</sup>	2.258 (2.061~2.561) <sup>*§</sup>	2.607 (2.152~2.901) <sup>*§#</sup>
dia 16 wk/dia 4 wk	EDL	1.234 (1.051~1.639)	1.045 (0.981~1.412) <sup>§</sup>	1.284 (1.033~1.625) <sup>#</sup>
	SOL	1.518 (1.216~1.921) <sup>*</sup>	1.268 (1.013~1.521) <sup>*§</sup>	1.828 (1.521~2.100) <sup>*§#</sup>
SOL/EDL	Con	0.790 (0.581~0.981)	0.963 (0.812~1.213) <sup>§</sup>	0.660 (0.459~0.994) <sup>#</sup>
	dia 4 wk	1.062 (0.995~1.412) <sup>Ⓢ</sup>	1.008 (0.876~1.213)	0.729 (0.527~0.983) <sup>§#</sup>
	dia 16 wk	1.306 (1.118~1.631) <sup>Ⓢ</sup>	1.222 (1.093~1.437) <sup>Ⓢ</sup>	1.039 (0.972~1.328) <sup>Ⓢ</sup>

The IC<sub>50</sub> ratio was defined as the IC<sub>50</sub> of twitch tension depression in one group or one muscle divided by the other group or the other muscle (e.g. IC<sub>50</sub> ratio=IC<sub>50</sub> (Dia4wk)/ IC<sub>50</sub> (Con), or IC<sub>50</sub> ratios=IC<sub>50</sub> (SOL in Con)/IC<sub>50</sub> (EDL in Con)). The twitch tension was elicited by indirect stimulation at 0.1 Hz. Values are expressed as means with 95% confidence intervals in IC<sub>50</sub> ratio (n=10 each). \*p<0.05 versus EDL, <sup>Ⓢ</sup>p<0.05 versus Con; <sup>Ⓢ</sup>p<0.05 versus Dia 4 wk; <sup>§</sup>p<0.05 versus Rocuronium, <sup>#</sup>p<0.05 versus Vecuronium. EDL, *Extensor digitorum longus*; SOL, *Soleus*; con, control group; dia 4 wk, diabetic 4 weeks group; dia 16 wk, diabetic 4 16 weeks group.

the control group (p<0.05; Fig. 3A~F). The curves were significantly shifted in those of the control group to the right in the diabetic groups in both muscles (p<0.05; Fig. 3). IC<sub>50</sub> was significantly larger in the diabetic groups in SOL for all the NDMRs and EDL for vecuronium and cisatracurium than the control group (p<0.05; Table 1), however, for rocuronium there was no significant difference between the dia 4 wk group and the control group in EDL (p>0.05; Table 1). For each NDMR, there was a significant difference between the curves in the dia 4 wk group and those in dia 16 wk group (p<0.05; Fig. 3A, B, D~F). But there was no significant difference between the curves in the dia 4 wk group and those in dia16wk group for cisatracurium in EDL (p>0.05; Fig. 3C). For rocuronium and cisatracurium in both SOL and EDL, IC<sub>50</sub> was significantly larger in dia 16 wk group than those in the dia 4 wk group (p<0.05; Table 1). For vecuronium in SOL, there was a significant difference between the IC<sub>50</sub> in the dia 4 wk group and those in dia16wk group for (p<0.05; Table 1), while no significant difference of the IC<sub>50</sub> values was found in the two groups for vecuronium in EDL (p>0.05; Table 1). The slopes of the concentration-twitch tension curves were larger in diabetic groups than that in control group (p<0.05; Table 1). The slope for rocuronium was significantly smaller than those for vecuronium and cisatracurium in the diabetic rats (p<0.05; Table 1). For rocuronium and vecuronium, the slope was significantly larger in the diabetic groups than those in the control group. For cisatracurium, there was no significant difference between the diabetic rats and the control ones (p>0.05; Table 1).

The IC<sub>50</sub> ratios, which standardized the magnitudes of the concentration-twitch tension curves, were defined as the IC<sub>50</sub> of twitch tension depression in one group or one muscle divided by the other group or the other muscle. Compared with control group, the IC<sub>50</sub> ratios in diabetic groups was largest for vecuronium, second largest for rocuronium, smallest for cisatracurium. The IC<sub>50</sub> ratios were significantly larger in SOL than in EDL for all the NDMRs (p<0.05; Table 2). For SOL/EDL, the IC<sub>50</sub> ratios were significantly largest in the dia 16 wk group, second largest in the dia 4 wk group, and smallest for the control (p<0.05; Table 2).

## DISCUSSION

The results of this study demonstrated that diabetes-induced desensitization to NDMRs depended on the stage of diabetes and on the different kind of muscles observed while was independent on different kind of NDMRs.

In previous studies, the hyposensitivity to NDMRs in diabetes has been testified, which was consistent with our findings in this report. In this study, the concentration-twitch tension curves of rocuronium, vecuronium, and cisatracurium were significantly different in the diabetic groups compared with the control group. And the IC<sub>50</sub> was significantly larger in the diabetic groups than the control group. Nerve conduction velocity is reduced in diabetic peripheral neurons, and pathogenetic mechanisms responsible for the impairment of axons and Schwann cells have been examined [4,23]. Axonal atrophy and demyelination, which was the signs of nerve degeneration, and the destabilization at NMJ in diabetic state may be the most probable explanation [4,24]. Nevertheless, the decrease of ACh release and the augmentation of presynaptic AChRs sensitivity, which due to the hypersensitivity to depolarizing muscle relaxant succinylcholine, has been shown [23,25]. All these controversial preclinical results may be relevant to the findings in clinical practice, in which delayed recovery or no alteration was determined [26,27]. Although current findings of resistance to muscle relaxants in diabetic rats was not in accord with that of succinylcholine or in human beings, different kinds of muscle relaxants, varied experiment conditions, *in vivo* or *in vitro*, different species should not be neglected. The slopes of the concentration-twitch tension curves, which indicated the affinities of AChRs, were larger in diabetic groups than that in control group [28]. The alteration in affinities of AChRs may occur in diabetic state. The similarity of nerve degeneration with denervation may induce the increased amount of AChRs. To the best of our knowledge, the distributions of muscle-type of AChRs are mainly restricted to the neuromuscular junction and activation of AChRs initiates contraction of skeletal muscle fibers by triggering endplate potentials at neuromuscular junctions [29]. AChRs appear over the entire muscle fiber surface, and fetal type AChR is expressed throughout the muscle fiber after denervation [30]. To achieve the same actions of neuromuscular block-

ing, the doses of NDMRs need to be increased to competitively bind to AChRs in diabetes. Further investigation should be done to testify the hypothesis.

Moreover, there was a significantly difference between the  $IC_{50}$  in the dia 4 wk group and those in dia 16 wk group for rocuronium and cisatracurium in both SOL and EDL in this study.  $IC_{50}$  was significantly larger in dia 16 wk group than those in the dia 4 wk group for vecuronium in SOL. Although there was no significantly difference between the  $IC_{50}$  values in the dia 4 wk group and those in dia 16 wk group for vecuronium in EDL,  $IC_{50}$  was larger in dia 16 wk group. In a word, regardless of the different muscle observed, diabetes induced the resistance to each NDMRs depends on the stage of diabetes for rocuronium, vecuronium and cisatracurium. The progress in muscle functions and fiber alteration occurs in a time-dependent manner [4]. In Brotto et al's report, the alteration in diaphragm contractility depended on the time after the onset of STZ-induced diabetes, which at least in part, relied on the switch in fiber type [12]. Although NMJs are the very early target in diabetes, the course of disease may play an important role in the final performance of changes in muscle contractility and alterations of response to NDMRs.

Compared with SOL in control group,  $IC_{50}$  in EDL was larger for rocuronium, vecuronium and cisatracurium than that in EDL. While in diabetic groups,  $IC_{50}$  in SOL was larger for every NDMRs. Additionally,  $IC_{50}$  ratio of SOL/EDL in dia 16 wk group was larger than that in dia 4 wk group. The results meant that the response to NDMRs depended on the muscles observed. STZ diabetes had differential effects on skeletal muscles accompanying the course of diabetes. It has been shown that diabetic SOL muscles had a higher proportion of slow-oxidative fibers than weight-matched controls while no obvious changes in fiber composition in diabetic EDL muscles [13]. For the slow SOL of the diabetic animals, muscle weight was preserved with respect to body weight. However, preferential atrophy of fast fibers in EDL was reflected in fiber area measurements [28,31]. Whereas SOL was protected from diabetic effects in terms of muscle mass and force production, both speed-related properties and oxidative capacity were impaired. In contrast, EDL underwent profound wasting and had decreased strength, while the speed-related properties and oxidative capacity was unchanged. The differences in the contractile and histochemical properties of slow and fast muscles may account for the differences in the resistance to NDMRs. The changes in fiber-type proportions of SOL which involved a change in muscle phenotypic expression, may induce more type I muscle fibers, which may result in much more resistance to NDMRs in SOL than that in EDL.

Compared with control rats, the  $IC_{50}$  in diabetic groups were different among the neuromuscular blockers. In addition, the  $IC_{50}$  ratios among the neuromuscular blockers were quite different with each other. However, there was no obvious relevance between the different kind of NDMRs and  $IC_{50}$  ratios. E. Narimatsu et al. concluded that the magnitude of sepsis-induced attenuation of potencies to NDMRs depended on the different molecular structures of neuromuscular blockers [28]. To the best of our knowledge, there are two kinds of NDMRs: one is benzyloisoquinolines (cisatracurium) and the other is bis-quaternary ammonium steroids (vecuronium and rocuronium). Our results demonstrated that diabetes-induced resistance to NDMRs was independent on the kind of NDMRs.

The results of our study seemed to be of great clinical relevance. Though the experiment was obtained *in vitro* by nerve-muscle preparations, the findings can be extrapolated to clinical studies. From the relevance in the conditions of different stages of diabetes between rat models and human beings, it is assumed that in clinical practice, the hyposensitivity to NDMRs may also develop to different extents during the course of diabetes. Moreover, now that diabetes-induced desensitization to NDMRs depended on the different kind of muscles observed, extra caution should be taken in clinical practice when monitoring for muscle relaxation in anesthetic management using different muscles. It is worth noticing that when choosing drugs for diabetic patients in anesthesia, NDMRs do not need further consideration as diabetes-induced resistance to NDMRs was independent on the kind of NDMRs.

In summary, diabetes-induced desensitization to NDMRs depended on the stage of diabetes and on the different kind of muscles observed while was independent on different kind of NDMRs. The resistance to NDMRs was stronger in the later stage of diabetes (16 weeks *versus* 4 weeks after STZ treatment in this report). Additionally, when monitoring in SOL, diabetes attenuated the actions of neuromuscular blockade than that in EDL. Nonetheless, the hyposensitivity to NDMRs in diabetes was not relevant for the kind of NDMRs.

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## REFERENCES

1. **Clements RS Jr.** Diabetic neuropathy--new concepts of its etiology. *Diabetes*. 1979;28:604-611.
2. **Diabetic peripheral neuropathies.** Physiopathology and clinical guidelines. *Diabetes Res Clin Pract*. 1986;2:183-256.
3. **Harati Y.** Diabetic peripheral neuropathies. *Ann Intern Med*. 1987;107:546-559.
4. **Fahim MA, Hasan MY, Alshuaib WB.** Early morphological remodeling of neuromuscular junction in a murine model of diabetes. *J Appl Physiol (1985)*. 2000;89:2235-2240.
5. **Marques MJ, Santo Neto H.** Acetylcholine receptors and nerve terminal distribution at the neuromuscular junction of non-obese diabetic mice. *Anat Rec*. 2002;267:112-119.
6. **Schofield GG, Furman BL, Marshall IG.** The effect of acute alloxan diabetes on the sensitivity of the rat skeletal neuromuscular junction to drugs. *Acta Diabetol Lat*. 1978;15:287-293.
7. **Minker E, Kac P, Blazsó G, Koltai M.** A study of the origin of altered pharmacological reactivity of synaptic structures caused by diabetes and pretreatment with contrainsular agents. *Acta Physiol Hung*. 1984;63:175-183.
8. **Kimura M, Fujihara M, Nojima H, Kimura I.** Hypersensitivity of acetylcholine receptor in diabetic skeletal muscle to neuromuscular blockers: the effect of myotubes cultured with spinal cord or its extract. *J Pharmacobiodyn*. 1986;9:29-38.
9. **Kimura M, Kimura I, Nojima H, Muroi M.** Diabetes mellitus-induced hypersensitivity of mouse skeletal muscles to acetylcholine and succinylcholine. *Jpn J Pharmacol*. 1986;40:251-256.
10. **Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, Stempel KE.** Metabolic profiles of three fiber types of skeletal muscle in

- guinea pigs and rabbits. *Biochemistry*. 1972;11:2627-2633.
11. **Cotter M, Cameron NE, Lean DR, Robertson S.** Effects of long-term streptozotocin diabetes on the contractile and histochemical properties of rat muscles. *Q J Exp Physiol*. 1989;74:65-74.
  12. **Brotto M, Brotto L, Jin JP, Nosek TM, Romani A.** Temporal adaptive changes in contractility and fatigability of diaphragm muscles from streptozotocin-diabetic rats. *J Biomed Biotechnol*. 2010;2010:931903.
  13. **Cotter M, Cameron NE, Lean DR, Robertson S.** Effects of long-term streptozotocin diabetes on the contractile and histochemical properties of rat muscles. *Q J Exp Physiol*. 1989;74:65-74.
  14. **Secher NH, Rube N, Secher O.** Effect of tubocurarine on human soleus and gastrocnemius muscles. *Acta Anaesthesiol Scand*. 1982;26:231-234.
  15. **Kitajima T, Ishii K, Kobayashi T, Ogata H.** Differential effects of vecuronium on the thumb and great toe as measured by accelography and electromyography. *Anaesthesia*. 1995;50:76-78.
  16. **Saitoh Y, Tanaka H, Toyooka H, Amaha K.** Recovery of post-tetanic and train-of-four responses at the first dorsal interosseous and adductor pollicis muscles in patients receiving vecuronium. *Can J Anaesth*. 1996;43:362-367.
  17. **Saitoh Y, Koitabashi Y, Makita K, Tanaka H, Amaha K.** Train-of-four and double burst stimulation fade at the great toe and thumb. *Can J Anaesth*. 1997;44:390-395.
  18. **Taquahashi Y, Yonezawa K, Nishimura M.** Influence of motor activities on the release of transmitter quanta from motor nerve terminals in mice. *J Vet Med Sci*. 1999;61:513-516.
  19. **Sieck DC, Zhan WZ, Fang YH, Ermilov LG, Sieck GC, Mantilla CB.** Structure-activity relationships in rodent diaphragm muscle fibers vs. neuromuscular junctions. *Respir Physiol Neurobiol*. 2012;180:88-96.
  20. **Wood SJ, Slater CR.** The contribution of postsynaptic folds to the safety factor for neuromuscular transmission in rat fast- and slow-twitch muscles. *J Physiol*. 1997;500:165-176.
  21. **McGuire M, MacDermott M.** The influence of streptozotocin diabetes and metformin on erythrocyte volume and on the membrane potential and the contractile characteristics of the extensor digitorum longus and soleus muscles in rats. *Exp Physiol*. 1999;84:1051-1058.
  22. **Oishi PE, Cholsiripunlert S, Gong W, Baker AJ, Bernstein HS.** Myo-mechanical analysis of isolated skeletal muscle. *J Vis Exp*. 2011;(48).
  23. **Kimura I, Okazaki M, Kimura M.** Streptozocin-diabetes modifies acetylcholine release from mouse phrenic nerve terminal and presynaptic sensitivity to succinylcholine. *Jpn J Pharmacol*. 1993;62:35-41.
  24. **Medina Sánchez M, Rodríguez Sánchez C, Vega Alvarez JA, Menéndez Peláez A.** Ultrastructural study of neuromuscular junction in rectus femoris muscle of streptozotocin-diabetic rats. *Histol Histopathol*. 1992;7:607-610.
  25. **Kimura M, Kimura I, Fujihara M, Hoshino N.** Diabetic state-induced modifications of succinylcholine binding mode in the microsomal fractions of mouse skeletal muscles. *Life Sci*. 1988;42:1029-1036.
  26. **Saitoh Y, Hattori H, Sanbe N, Nakajima H, Akatu M, Murakawa M.** Reversal of vecuronium with neostigmine in patients with diabetes mellitus. *Anaesthesia*. 2004;59:750-754.
  27. **Alper I, Ulukaya S, Makay O, Balcioglu T.** The pharmacodynamic effects of rocuronium during general anesthesia in patients with type 2 diabetes mellitus. *Minerva Anesthesiol*. 2010;76:115-119.
  28. **Narimatsu E, Niiya T, Kawamata M, Namiki A.** Sepsis stage dependently and differentially attenuates the effects of nondepolarizing neuromuscular blockers on the rat diaphragm in vitro. *Anesth Analg*. 2005;100:823-829.
  29. **Lee BH, Shin TJ, Hwang SH, Choi SH, Kang J, Kim HJ, Park CW, Lee SH, Nah SY.** Inhibitory effects of quercetin on muscle-type of nicotinic acetylcholine receptor-mediated ion currents expressed in *Xenopus* Oocytes. *Korean J Physiol Pharmacol*. 2011;15:195-201.
  30. **Salpeter MM, Loring RH.** Nicotinic acetylcholine receptors in vertebrate muscle: properties, distribution and neural control. *Prog Neurobiol*. 1985;25:297-325.
  31. **McGuire M, MacDermott M.** The influence of streptozotocin diabetes and metformin on erythrocyte volume and on the membrane potential and the contractile characteristics of the extensor digitorum longus and soleus muscles in rats. *Exp Physiol*. 1999;84:1051-1058.