

Tetanic fade induced by d-Tubocurarine, Hexamethonium and Neostigmine in phrenic nerve diaphragm of diabetic rats

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ABSTRACT. The acetylcholine released from motor nerve terminal (MNT) can regulate its own output (MNT automodulation) acting on nicotinic (positive feed back automodulation) or muscarinic (negative feedback automodulation) presynaptic receptors. On the other hand, diabetic neuropathy, a disorder of peripheral nerves, is one of the most common complication of diabetes mellitus that produces serious alterations on motor nerve terminal without interfering in the velocity and integrity of neuro-muscular transmission. Pharmacological studies have shown that diabetic animals are less sensitive to some neuromuscular blockades such as d-tubocurarine, galamine, pancuronium or decamethonium than normal ones. Results suggest that change in MNT automodulation may counterbalance neuronal deficiencies induced by diabetes. The present study with phrenic nerve-diaphragm preparations from normal and diabetic animals was conducted to verify whether differences in neuro-muscular fade induced by d-tubocurarine, hexamethonium or neostigmine exist. Results showed that there were no differences in neuro-muscular fade induced by neostigmine, hexamethonium or d-tubocurarine. However, the recovery of tetanic fade induced by d-tubocurarine was faster in preparations obtained from diabetic rats. Difference might be explained by decrease in affinity of d-tubocurarine for presynaptic nicotinic receptors.

Key words: diabetes mellitus, d-tubocurarine, hexamethonium, neostigmine, tetanic fade, Wedensky inhibition.

RESUMO. Fadiga neuromuscular induzida por d-Tubocurarina, Hexametônio e Neostigmina em preparações nervo frênico-diafragma isolado de ratos diabéticos.

A acetilcolina liberada do terminal nervoso motor (TNM) pode modular sua própria liberação (automodulação do TNM), interagindo com receptores nicotínicos (autoestimulação do TNM) ou muscarínicos (autoinibição do TNM) pré-juncionais. Por outro lado, tem-se demonstrado que a neuropatia induzida pelo estado diabético determina vários danos estruturais no interior do TNM, sem, contudo, interferir na velocidade e na integridade da transmissão neuromuscular. Estudos farmacológicos demonstram que animais diabéticos, quando comparados aos normais, são menos sensíveis a alguns bloqueadores neuromusculares (d-tubocurarina, galamina, pancurônio e decametônio). Esses resultados sugerem que alguma modificação no sistema de automodulação do TNM pode contrabalançar as deficiências neuronais induzidas pelo estado diabético. Dessa forma, o presente estudo foi conduzido com preparações nervo frênico-diafragma isolado de ratos (obtidas de animais normais e diabéticos) na tentativa de verificar se existiriam diferenças na fadiga neuromuscular induzida por drogas (d-tubocurarina, neostigmina, hexametônio). Nossos resultados mostraram que, embora não existissem diferenças na indução da fadiga neuromuscular induzida por d-tubocurarina, neostigmina ou hexametônio, o recobro da fadiga neuromuscular induzida por d-tubocurarina foi mais rápido em preparações neuromusculares obtidas de animais diabéticos. Essa diferença pode estar relacionada a alguma modificação induzida pelo estado diabético que determinou redução da afinidade da d-tubocurarina para os receptores nicotínicos pré-juncionais.

Palavras-chave: diabetes mellitus, d-tubocurarina, fadiga neuromuscular, hexametônio, inibição de Wedensky, neostigmina.

Skeletal muscles respond with sustained contractions when electrically stimulated at physiological frequencies (30-80 Hz), but tetanic fade (=Wedensky inhibition) may be observed when

the frequencies of stimulation about 100 Hz. are applied on motor nerve (Alves-do-Prado *et al.*, 1987; Bowman, 1980). Tetanic fade may also be observed during physiologic frequencies of stimulation

applied on motor nerve if muscle is treated with neostigmine (Alves-do-Prado *et al.*, 1987), d-tubocurarine, or hexamethonium (Alves-do-Prado *et al.*, 1987; Bowman, 1980). Theoretical models suggest that acetylcholine, besides acting on subsynaptic membranes, acts on prejunctional cholinergic receptors too to change the acetylcholine mobilization process and thus to control the neurotransmitter output during tetanic stimulation (automodulation of the motor nerve terminal) (Alves-do-Prado *et al.*, 1987; Bowman, 1980, 1990). Nicotinic and muscarinic sites exist on motor nerve endings (Bowman, 1990). The blockade of nicotinic presynaptic receptors by d-tubocurarine or hexamethonium induces Wedensky inhibition which is antagonized by atropine (Alves-do-Prado *et al.*, 1987). Such inhibition is produced by action of the acetylcholine on inhibitory presynaptic muscarinic receptors (Alves-do-Prado *et al.*, 1987). Whereas the anticholinesterase agents, such as neostigmine, also induce Wedensky inhibition antagonized by atropine, the fade is produced by acetylcholine action on nicotinic and muscarinic presynaptic receptors (Alves-do-Prado *et al.*, 1987).

Diabetic neuropathy, a disorder of peripheral nerves, is one of the most common complications of diabetes mellitus (Brown and Asbury, 1984) that produces serious alterations on motor nerve terminal including mitochondrial degeneration, breakdown of presynaptic membrane as well as changes in form of the synaptic vesicles (Sánchez *et al.*, 1992). Electrophysiological study has shown that the number of failures of neuronal action potential as well as the number of failures of neuromuscular transmission, are lower in neuromuscular preparations from diabetic rats than in those from normal animals (Schiller and Rahamimoff, 1989), even though in neuromuscular preparation from diabetic animals the quantal content and the amplitude of the end plate potential are reduced (Constantini *et al.*, 1987). On the other hand, pharmacological studies have shown that neuromuscular blockade induced by d-tubocurarine, galamine, pancuronium or decamethonium is smaller in neuromuscular preparations from diabetic animals than that in normal ones (Minker *et al.*, 1986). Since the study performed with autonomic ganglions of diabetic animals did not show any correlation between the alteration of the pharmacological reactivity and blood sugar level (Minker *et al.*, 1986) and changes in cholinesterase activity (Minker *et al.*, 1978), it is possible that diabetes mellitus induces changes on automodulation system, such as an increase in its efficiency. The present work was conducted to verify whether or not the tetanic fade induced by d-tubocurarine, hexamethonium or neostigmine is

different in neuromuscular preparations from normal and diabetic rats.

Materials and Methods

Neuromuscular preparation. Phrenic nerve-diaphragm muscles were isolated from normal and diabetic streptozotocin-induced Wistar rats and assembled according to the method described by Bulbring (1946). Each muscle was immersed in a 30-ml chamber containing nutrient solution (NaCl, 188; KCl, 47; CaCl₂, 1.9; MgSO₄, 1.2; NaHCO₃, 25; KH₂PO₄, 1.2 and glucose, 11 concentration in mmol/l) at 37°C and continuously aerated with a mixture of oxygen (95%) and carbon dioxide (5%). Stimulation of the phrenic nerve was achieved with a bipolar platinum electrode using supramaximal rectangular pulses (0.2 or 100 Hz, 0.05 ms). Muscular contractions were recorded on a smoked drum by means of a Starling-type lever.

Induction of diabetes, experimental design and data analysis. Diabetes was induced in male rats, weighing about 250 mg, by a single administration of streptozotocin (35 mg/Kg) into the penial vein. The streptozotocin was dissolved in citrate buffer (10 mM and pH 4.5). After four weeks the diabetic animals with glucose blood level about 200 mM and control animals (buffer citrate) were used in the experiments.

In all experiments the standard rate of stimulation was 0.2 Hz; at 5 min intervals there was stimulation of 100 Hz (tetanic stimulation) applied to the nerve for 10 sec. The tension produced at the beginning of tetanic stimulation (A) was compared to that obtained at the end of tetanic stimulation (B) and the ratio ($R = B/A$) was performed. The drugs (hexamethonium, d-tubocurarine and neostigmine) were added in the bath at $t = 0$ and tetanic stimulation was then repeated at 5-min intervals for 60 min. One minute after tetanic frequency of stimulation applied to nerve at $t = 10$ min, the nutrient solution containing drug was exchanged for drug-free nutrient solution (4 X). The same procedure was adopted to the subsequent tetanic frequency applied to the nerve. The R values obtained after drugs addition were compared to the R values obtained with drug free nutrient solution. Student's "t" test ($p < 0.05$) was performed to compare the data obtained with use of neuromuscular preparations obtained from diabetic rats with those from normal ones.

Results

The tetanic fade induced by 100 Hz applied to the motor nerve did not produce different R values to the preparation obtained from normal or diabetic animals (Figure 1). The induction and recovery of tetanic fade produced by hexamethonium (130.0 μ M) and

neostigmine ($2.0 \mu\text{M}$) were not different in neuromuscular preparations obtained from diabetic or non-diabetic animals (Figures 2 and 3). The d-tubocurarine ($14 \mu\text{M}$) fade-induced was also similar to neuromuscular preparations from normal or diabetic animals, but the recovery of tetanic fade was better when the studies were performed with neuromuscular preparations from diabetic than from normal animals (Figure 4).

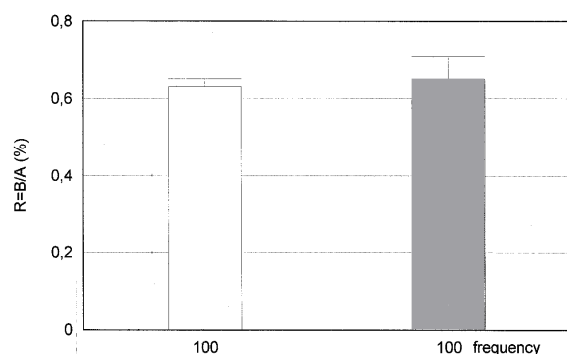


Figure 1. % of Wedensky inhibition (R) (see text for detail) induced through 100 Hz applied on phrenic nerve of neuromuscular preparations obtained from normal (white columns) and diabetics (dark columns) rats when the solution nutrient was drug-free. Height of columns indicate media \pm SD of 15 preparations

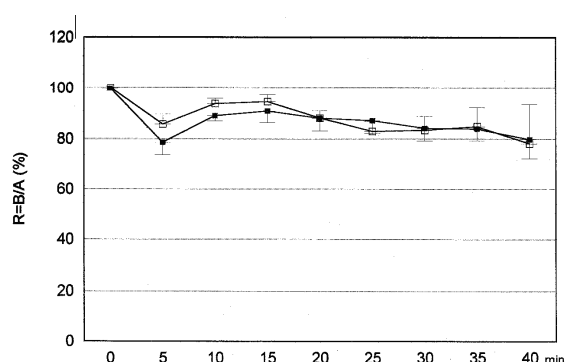


Figure 2. % of Wedensky inhibition (R) (see text for detail) induced by hexamethonium ($130.0 \mu\text{M}$) in neuromuscular preparation obtained from normal (○) and diabetics (■) rats. Symbols indicate media \pm SD of 8 to 10 preparations. Abscissas indicate time in which 100 Hz was applied

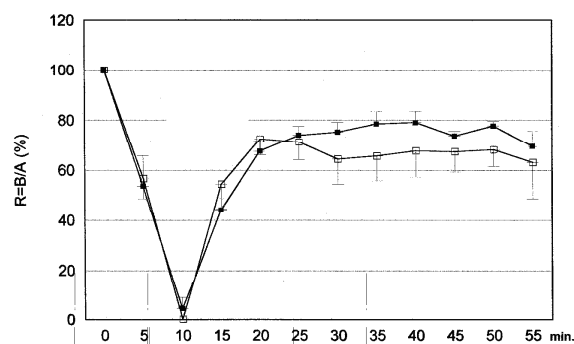


Figure 3. % of Wedensky inhibition (R) (see text for detail) induced by neostigmine ($2.0 \mu\text{M}$) in neuromuscular preparation obtained from normal (○) and diabetics (■) rats. Symbols indicate media \pm SD of 10 to 12 experiments. Abscissas indicate time in which 100 Hz was applied

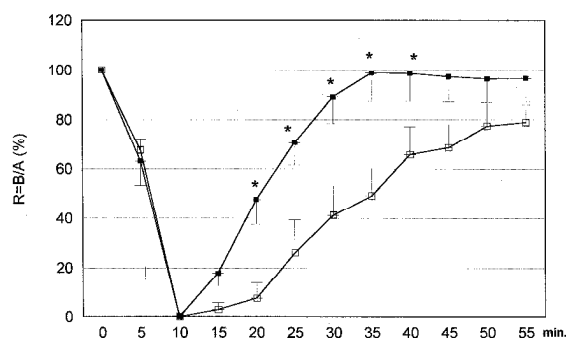


Figure 4. % of Wedensky inhibition (R) (see text for detail) induced by d-tubocurarine ($14.0 \mu\text{M}$) in neuromuscular preparation obtained from normal (○) and diabetics (■) rats. Symbols indicate media \pm SD of 10 to 12 preparations. Abscissas indicate time in which 100 Hz was applied

Discussion

The d-tubocurarine- or hexamethonium-induced Wedensky inhibition is produced by blockade of presynaptic nicotinic receptors (positive feedback modulation) and stimulation of presynaptic muscarinic receptors (negative feedback modulation) determining reduction of acetylcholine released from motor nerve terminal (Alves-do-Prado *et al.*, 1987). On the other hand, the neostigmine-induced neuromuscular fade may be produced by stimulation in nicotinic and muscarinic presynaptic receptors increasing the acetylcholine released (nicotinic positive feedback modulation) and generating an intense stimulation of inhibitory presynaptic muscarinic receptors (negative feedback automodulation) (Alves-do-Prado *et al.*, 1987).

In studies performed with drug-free nutrient solution, the R values produced by 100 Hz applied on motor nerve were similar in preparations obtained from normal or diabetic animals. Since serious alterations on motor nerve terminal have been observed in diabetics animals (Sánchez *et al.*, 1992), result suggests compensatory mechanism acting on neuromuscular transmission of the preparations obtained from diabetic rats.

Results also showed that there were no differences between the fade induced by neostigmine, hexamethonium or d-tubocurarine in the studies performed with neuromuscular preparation from normal and diabetic rats, but the recovery (after nutrient exchanged) of Wedensky inhibition induced by d-tubocurarine was faster in the preparations from diabetic rats than from normal ones. Results obtained from diabetic rats suggest that Wedensky inhibition induced by hexamethonium, neostigmine or d-tubocurarine seem to follow the same mechanism of action described above on neuromuscular preparation from normal animals. Since Wedensky inhibition

is related to nicotinic and muscarinic presynaptic sites (Alves-do-Prado *et al.*, 1987; Bowman, 1990, 1980), the difference observed in the recovery (after nutrient solution exchanged) of Wedensky inhibition induced by d-tubocurarine might be explained through smaller affinity of d-tubocurarine at nicotinic receptors on motor nerve terminal of preparations from diabetic rats. This hypothesis is supported by results obtained by hexamethonium, a blocker of nicotinic receptor with curare-like action, that produced recovery of tetanic fade similar to neuromuscular preparations from diabetic and normal animals. From neuromuscular preparations from diabetic animals it has been shown that stimulatory nicotinic presynaptic receptors are highly sensitive to succinylcholine (Kimura *et al.*, 1993). Thus, it is possible that alteration on presynaptic nicotinic receptors of neuromuscular preparation from diabetic animals (Kimura *et al.*, 1993) produced reduction on its affinity for d-tubocurarine, without modifying its affinity for hexamethonium. As a conclusion one may state that the diabetic state may reduce the affinity of nicotinic presynaptic receptor at d-tubocurarine.

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