

Reversal by methylene blue of tetanic fade induced in cats by nitric oxide

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Abstract

Previous data from our laboratory have indicated that nitric oxide (NO) acting at the presynaptic level increases the amplitude of muscular contraction (AMC) of the phrenic-diaphragm preparations isolated from indirectly stimulated rats, but, by acting at the postsynaptic level, it reduces the AMC when the preparations are directly stimulated. In the present study we investigated the effects induced by NO when tetanic frequencies of stimulation were applied to *in vivo* preparations (sciatic nerve-anterior tibial muscle of the cat). Intra-arterial injection of NO (0.75-1.5 mg/kg) induced a dose-dependent increase in the Wedensky inhibition produced by high frequencies of stimulation applied to the motor nerve. Intra-arterial administration of 7.2 µg/kg methylene blue did not produce any change in AMC at low frequencies of nerve stimulation (0.2 Hz), but antagonized the NO-induced Wedensky inhibition. The experimental data suggest that NO-induced Wedensky inhibition may be mediated by the guanylate cyclase-cGMP pathway.

Key words

- Wedensky inhibition
- Nitric oxide
- Methylene blue
- Skeletal muscle

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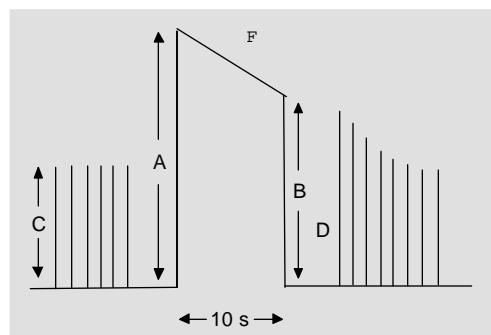
Neuromuscular fade (tetanic fade or Wedensky inhibition) in myographic records is a poorly sustained contraction that follows a fast muscular contraction of high amplitude when the motor nerve is receiving high electrical frequencies of stimulation (see Figure 1). Skeletal muscles respond with sustained contractions when the nerve is stimulated at physiological frequencies (30-80 Hz), but Wedensky inhibition may be achieved when frequencies of stimulation higher than 100 Hz are applied to the motor nerve (1,2).

Studies on vascular smooth muscle have shown that vasodilatation is endothelium dependent and mediated by the nitric oxide (NO)-synthase-guanylate cyclase-cGMP pathway (3-5). A similar pathway has been demonstrated in rat skeletal muscle (6) where NO may act at the pre-and postsynaptic lev-

els (7). Its action at the presynaptic level increases the amplitude of muscular contraction (AMC) when the muscle is indirectly stimulated, but its action at the postsynaptic level induces an increase followed by a reduction in AMC when the preparations are directly stimulated (7). The effects of NO have not been studied in *in vivo* neuromuscular preparations submitted to high rates of stimulation of the motor nerve. Since the neuromuscular preparations from cats are useful for *in vivo* studies using high frequencies of stimulation of the motor nerve (1,2), the present study was undertaken to determine the effects of NO on Wedensky inhibition in cat anterior tibial muscle preparation.

The preparations used in the present study have been described in detail in a previous report (2). Briefly, the popliteal artery was

Figure 1- Examples of cat anterior tibial muscle contractile response during tetanic stimulation. The tetanic fade is calculated as the ratio (R) between the tension at the end (B) and the tension at the beginning (A) of the tetanic response ($R = B/A$). C and D correspond to pretetanic and post-tetanic twitches, respectively. F represents the rate of stimulation required to obtain $R = 0.75$.



exposed and all branches except those to the anterior tibial muscle and the middle genicular artery were tied off. For intra-arterial drug injection, a polyethylene cannula was introduced into the middle genicular artery and tied close to the popliteal artery. During intra-arterial injection of the drugs, the anterior tibial artery was occluded just below the entrance of its branch into the anterior tibial muscle. The contractions of the tibial muscle were evoked by electrical stimulation of the sciatic nerve and recorded on an Ugo Basile polygraph. In all experiments the standard rate of stimulation was 0.2 Hz, but stimulation at a higher (tetanic) rate was applied to the nerve for 10 s at 10-min intervals. The tension produced at the

beginning of tetanic stimulation (A) was compared to that obtained at the end of tetanic stimulation (B) (Figure 1). The stimulation rate (F) (100-120 Hz) required to obtain a 0.75 ratio ($R = B/A$) was determined for each preparation and was used throughout the experiment. Saline (0.05 ml) was injected intra-arterially (time $t = 0$) and F was applied to the nerve 5 min later. The smallest dose of NO that reduced the tension ratio (R) values to about 85% of the R control was determined and injected at $t = 10$ min and tetanic stimulation was then repeated at $t = 15, 25, 35$ and 45 min. The same sequence was repeated 60 min later with saline containing the smallest effective dose of methylene blue. The anterior tibial artery was freed at $t = 16$ min. Variations in R are reported as percent R obtained at $t = 5$ min. Data were analyzed by the Student *t*-test, with the level of significance set at $P < 0.05$. NO was obtained from sodium nitrite (8).

The experiments showed that intra-arterial injection of NO (0.75-1.5 mg/kg) induced a dose-dependent increase in Wedensky inhibition when high frequencies were applied to the nerve 5 min after drug administration

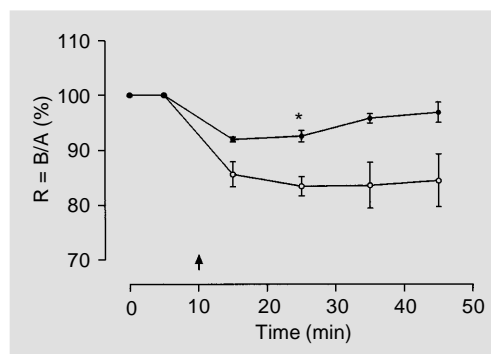


Figure 2 - Tetanic fade induced by 0.75 (filled circles) and 1.5 mg/kg (open circles) nitric oxide (NO) in cat anterior tibial muscle preparations. On the ordinate, the R ratio (B/A) is expressed as a percentage of that obtained 5 min after injection of drug-free saline. NO was injected at time = 10 min (arrow). Drugs were administered through the middle genicular artery. Points represent the mean (\pm SEM) of 4 to 6 experiments. * $P < 0.05$ compared to the R ratio obtained with the intra-arterial injection of 1.5 mg/kg NO.

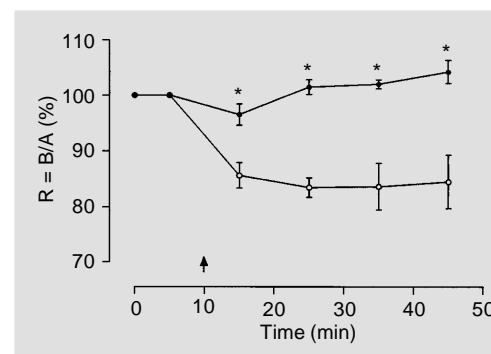


Figure 3 - Antagonism by methylene blue (7.2 μ g/kg) of tetanic fade induced by nitric oxide (NO) (1.5 mg/kg) in cat anterior tibial muscle preparations. On the ordinate, the R ratio (B/A) is expressed as a percentage of that obtained 5 min after injection of drug-free saline (open circles) or saline containing methylene blue (filled circles). NO was injected at time = 10 min (arrow). Drugs were administered through the middle genicular artery. Points represent the mean (\pm SEM) of 4 to 6 experiments. * $P < 0.05$ compared to % of the R ratio obtained with the use of 1.5 mg/kg NO in the absence of methylene blue.

(Figure 2). The smallest dose of NO that reduced R values to about 85% was 1.5 mg/kg. The same doses of sodium nitrite or the same volume of acidic solution (pH = 1.0) or saline did not induce any change in R (data not shown). NO (0.75-1.5 mg/kg)-induced fade remained below the control level for more than 30 min (Figure 2). Intra-arterial administration of 7.2 µg/kg methylene blue did not produce any change in AMC when low frequencies (0.2 Hz) were applied to the nerve (data not shown), but antagonized the NO-induced Wedensky inhibition (Figure 3). These results show for the first time Wedensky inhibition induced by NO as well

as its antagonism by methylene blue. The experimental data also suggest that NO-induced Wedensky inhibition is mediated by the guanylate cyclase-cGMP pathway since the methylene blue doses used in the present study were lower than those used to inhibit NO-synthase activity (9).

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