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Effects of L-arginine on the diaphragm muscle twitches elicited at different frequencies of nerve stimulation

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Abstract

In rats, the nitric oxide (NO)-synthase pathway is present in skeletal muscle, vascular smooth muscle, and motor nerve terminals. Effects of NO were previously studied in rat neuromuscular preparations receiving low (0.2 Hz) or high (200 Hz) frequencies of stimulation. The latter frequency has always induced tetanic fade. However, in these previous studies we did not determine whether NO facilitates or impairs the neuromuscular transmission in preparations indirectly stimulated at frequencies which facilitate neuromuscular transmission. Thus, the present study was carried out to examine the effects of NO in rat neuromuscular preparations indirectly stimulated at 5 and 50 Hz. The amplitude of muscular contraction observed at the end (B) of a 10-s stimulation was taken as the ratio (R) of that obtained at the start (A) (R = B/A). S-nitroso-N-acetylpenicillamine (200 µM), superoxide dismutase (78 U/ml) and L-arginine (4.7 mM), but not D-arginine (4.7-9.4 mM), produced an increase in R (facilitation of neurotransmission) at 5 Hz. However, reduction in the R value (fade of transmission) was observed at 50 Hz. N^G-nitro-L-arginine (8.0 mM) antagonized both the facilitatory and inhibitory effects of L-arginine (4.7 mM). The results suggest that NO may modulate the release of acetylcholine by motor nerve terminals.

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- S-nitroso-N-acetylpenicillamine
- Superoxide dismutase

Nitric oxide (NO) is synthesized by NO-synthase (NOS) (1-3). In the rat, NOS is present in the sarcolemma of type II fibers of skeletal muscle (4), in vascular smooth muscle (5), and in motor nerve terminals (6). Since NOS is a stereospecific enzyme, the effects induced by L-arginine are not observed in the presence of D-arginine under similar experimental conditions (7,8). Several analogues of L-arginine (N^G-monomethyl L-arginine; N^G-nitro-L-arginine (L-NOARG); L-arginine methyl ester; L-arginine ethyl ester, and N^G-nitro-L-arginine methyl ester) are used as inhibitors of NOS. The effects produced by endogenous NO (from L-arginine) are pharmacologically similar to those induced by NO released from an exogenous source such as 3-(4-morpholinyl)-syndonone imine (SIN-1) or S-nitroso-N-acetylpenicillamine (SNAP) (9).

The amplitude of muscular contraction (AMC) is stable when the diaphragm is indirectly stimulated at 0.2 Hz. A progressive increase in AMC is observed at 5 Hz (see

Figure 1 for illustration) and tetanic contraction is observed at 50 Hz (Figure 1).

Acting at the presynaptic level, the NO precursor L-arginine (4.7-9.4 mM) produces a dose-dependent increase of AMC in rat neuromuscular preparations indirectly stimulated at 0.2 Hz (10). In contrast, acting on skeletal muscle, it reduces AMC in preparations previously paralyzed with d-tubocurarine and directly stimulated at 0.2 Hz (10). The presynaptic action of NO reduces the effect produced by its postsynaptic action (10). On the other hand, the NO precursor L-arginine (4.7 to 9.4 mM) or SIN-1 acting at the presynaptic level produces a dose-dependent increase in tetanic fade when the nerve is stimulated at 200 Hz (11). The same high frequency of stimulation reduces the maximal tetanic tension (postsynaptic action) when applied to the rat phrenic nerve diaphragm preparation previously treated with L-arginine or SIN-1 (11). Similar results have been observed when assays using high frequency are performed with superoxide dismutase (SOD), a selective scavenger of superoxide anion radicals (11). Since in previous studies increase or reduction of neuromuscular transmission by NO was not investigated in preparations indirectly stimulated at frequencies which facilitate neuromuscular transmission, in the current study we examine the effects of NO in rat neuromuscular preparations indirectly stimulated at frequencies which induce an increase in AMC (5 Hz) or produce tetanic contraction (50 Hz).

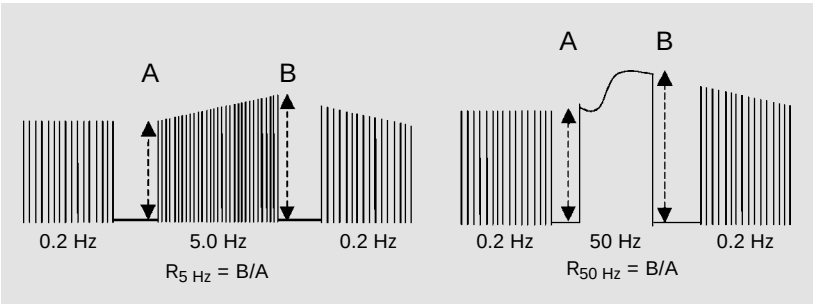

Figure 1. Diagram representation of muscular contraction induced by different frequencies of stimulation applied indirectly to the rat diaphragm. Drug effects are calculated as the ratio (R) between the tension at the end (B) and the maximal tension at the start (A) (R = B/A) of the response recorded at different frequencies of stimulation (5 and 50 Hz). The maximal tetanic tension is 15 g at 5 Hz and 22 g at 50 Hz. The sensitivity of the recording system at 50 Hz is lower than at 5 Hz and the latter lower than at 0.2 Hz.

Phrenic nerve and diaphragm muscles were isolated from Wistar rats by the method of Bülbring (12). Each muscle was immersed in a 20-ml chamber containing Krebs buffer (188 mM NaCl, 4.7 mM KCl, 1.9 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, and 11 mM glucose) at 37°C and continuously aerated with a mixture of oxygen (95%) and carbon dioxide (5%). The phrenic nerve was stimulated with a bipolar platinum electrode using a supramaximal rectangular pulse (0.2 Hz, 0.05 ms). Isometric muscular contractions were recorded on an Ugo Basile polygraph (Ugo Basile, Varese, Italy).

In studies performed at 5 Hz, the isolated muscles were stimulated at 0.2 Hz until a steady amplitude of muscle contraction was obtained. The lowest concentrations of L-arginine, SNAP and SOD capable of producing effects were determined and then added to the organ bath (t = 0 min). Stimuli of 5 and 50 Hz were applied to the motor nerve for 10 s at 15-min intervals. The tension produced at the end of stimulation (B) was compared as a ratio (R) of tension at the start (A) (R = B/A) (Figure 1). After L-arginine, D-arginine, SNAP or SOD addition, 5 and 50 Hz stimulation was repeated at t = 20 min. The same sequences were repeated with the use of L-NOARG, but, in this case, the NOS inhibitor was added 15 min before L-arginine. R obtained after drug addition was taken as a percentage of that observed before any drug administration. The unpaired Student *t*-test was used for data comparison with the level of significance set at $P<0.05$.

L-arginine (4.7 mM) increased the R value at 5 Hz, but reduced it at 50 Hz. The L-arginine-induced effects were stereospecific, since D-arginine (4.7-9.4 mM) was not effective in similar experiments. L-arginine-induced effects were reduced by L-NOARG

(8.0 mM). Since L-NOARG and L-arginine are taken up by the cell through different carrier systems (13), these results suggest that the L-arginine-induced effects depend on its metabolism to NO. This hypothesis is reinforced by SNAP results (200 µM) which were similar to those induced by the amino acid (Figure 2).

The selective removal of superoxide anion radicals by SOD (78 U/ml) also induced an effect similar to that of L-arginine at 50 Hz (Figure 2). However, the same agent did not produce any effect at 5 Hz. This difference may depend on both the metabolism of L-arginine producing additional NO in the tissues and its ability to scavenge superoxide anions, thereby increasing NO bioavailability (14).

The lowest concentrations of L-arginine, SNAP, SOD and L-NOARG found in the current study were 4.7 mM, 200 µM, 78 U/ml and 8.0 mM, respectively. Although the lowest concentration of L-arginine was similar to that obtained in previous studies at 0.2 Hz (10), the lowest concentration of L-NOARG was higher than 4.7 mM, which was determined in similar studies performed at 0.2 Hz (10). These observations suggest that NOS activity may depend on the frequency of stimulation applied to the motor nerve.

In cats it has been shown that tetanic fade induced by NO depends on the action of gas at the presynaptic level; it might increase acetylcholine release from motor nerve terminals, with a consequent activation of inhibitory presynaptic muscarinic receptors (15). Such mechanism might explain the reduction in R values obtained with NO at 50 Hz, but not the increase in R values recorded with NO at 5 Hz.

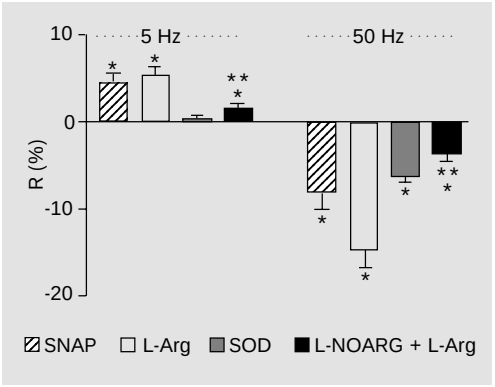

Figure 2. Percentage of increase and reduction in R values induced by S-nitroso-N-acetylpenicillamine (SNAP, 200 µM), L-arginine (L-Arg, 4.7 mM), and superoxide dismutase (SOD, 78 U/ml) in rat neuromuscular preparations indirectly stimulated with 5 Hz (left columns) and 50 Hz (right columns). Black columns represent the effects induced by L-Arg after N^G-nitro-L-arginine (L-NOARG, 8.0 mM) addition. On the ordinate, ratio R (see Figure 1) is expressed as a percentage of that obtained with drug-free Krebs buffer, taken as 100% (t = 0). The abscissa represents frequency values in Hz. Column height represents the mean (± SEM) of 6 to 8 experiments. *P<0.05 compared to control; **P<0.05 compared to L-Arg in the absence of L-NOARG (Student *t*-test).

It is known that the release of acetylcholine from motor nerve terminals can also be modulated by endogenous agents other than acetylcholine, such as adenosine, catecholamines, metabolic arachidonic acid, calcitonin gene-related peptide, substance P, VIP, and hormones (16,17). Thus, the present study shows that NO may possibly represent another modulating factor of acetylcholine release by the motor nerve terminal.

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