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THE INFLUENCE OF LIDOCAINE AND BETA-BLOCKERS ON L-NAME-INDUCED POTENTIATION OF THE ISOLATED RAT HEMIDIAPHRAGM CONTRACTILITY

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L-NAME (1 - 10 mM, 30 min of incubation) potentiates the tension developed (Td) in the isolated rat hemidiaphragm stimulated by direct subtetanic electrical stimulation (DSES) (F = 14 Hz). The aim of our study was to investigate the possible modulatory effect of local anaesthetic (lidocaine) and beta adrenergic blockers, propranolol and atenolol, on the L-NAME- induced potentiation of isometric contractility (IC). Lidocaine (0.1 mM, 5 min incubation) did not change Td by itself. Also, in the presence of lidocaine (0.1 mM, 5 min incubation), the stimulatory effect of L-NAME (3 mM, 30 min incubation) was preserved. In contrast, lidocaine itself (0.1 mM, 30 min incubation) produced an increase of Td up to 50%. The incubation of the muscle with both lidocaine (0.1 mM, 30 min incubation) and L-NAME (3 mM, 30 min incubation) produced an almost additive stimulatory effects, up to 90%, on Td. Propranolol itself (1 μM, 5 min incubation) did not change Td. In the presence of propranolol, L-NAME maintained the stimulatory effect on Td. Propranolol (1 μM, 30 min incubation) did not change Td itself, but abolished the stimulatory effect of L-NAME. Atenolol (1 μM, 30 min incubation) neither significantly changed Td nor L-NAME-induced potentiation of Td.

Key words: aminophylline, beta-blockers, lidocaine, L-NAME, rat diaphragm

INTRODUCTION

Both constitutive and inducible nitric oxide (NO) synthase isoforms have been identified in skeletal muscles: type I (neuronal costitutive NOS – ncNOS), type III (endothelial constitutive NOS – ecNOS) and type II (inducible NOS – iNOS) (Förstermann and Kleinert, 1995). Activity of both constitutive isoforms is regulated by calcium ions. In contrast, NOS II is a Ca^{2+} – independent isoform, and its activity can be induced by cytokines and other inflammatory mediators (Reid, 1998). It was shown that L-arginine-derived NO, modulates the contractility and metabolism of isolated skeletal muscles (Kobzik *et al*., 1994). The neuronal isoform of NOS (nNOS), most evident in fast fibres, has a major role in skeletal

muscle contractility (Grozdanovic *et al.* 1997). The activity of nNOS can be upregulated during repetitive isometric contractions of skeletal muscles. Therefore, resting rat diaphragm produces approximately 3-5 pmol NO equivalents/mg/min of NO, while during active contractions, diaphragm NO production is increased approximately sixfold (Stamler and Meissner, 2001). Consequently, the influence of NO and NOS inhibitors on muscle contractillity were extensively investigated during the last decade (Kobzik *et al*., 1994; Reid 1998; Stojanović *et al*., 2003; Stojanović *et al*., 2004). It was postulated that NO originated by nNOS, modulates the contractile function of skeletal muscles *via* cGMP patway and/or directly (S-nitrosylation of sarcoplasmatic calcium ryanodine receptor and/or Ca-ATPase) (Reid, 1998).

The interaction between aminophylline (AMPh) and L-NAME (a potent inhibitor of constitutive isoforms of NOS) on the contractility of isolated rat diaphragm stimulated by direct subtetanic electrical stimulation, as well as the role of extracellular calcium in such interactions were investigated in our laboratory (Stojanović *et al*., 2003; Stojanović *et al*., 2004). It was of interest to assess whether such interactions are modulated by sarcolemmal changes induced by lidocaine, as well as the role of beta adrenergic receptors in such interactions.

MATERIALS AND METHODS

The experiments were performed on isolated rat hemidiaphragm. Wistar rats of both sexes (200-250 g) were bred and kept under standard laboratory conditions. The investigation conforms to the Guide for the care and use of laboratory animals published by the US National Institute of Health (NIH publication No. 85-23, revised 1985).

The hemidiaphragms from male and female rats were suspended in an isolated organ bath of 20 ml capacity. The muscle was immersed in Tyrode solution with a double amount of glucose (11.1 mM) and bubbled with a mixture of 97% O_2 and 3% CO_2. The Tyrode solution composition was as follows: 136 mM NaCl, 2.81 mM KCl, 0.105 mM $MgCl_2$, 1.08 mM $CaCl_2$, 0.417 mM NaH_2PO_4, 11.9 mM $NaHCO_3$ and 11.1 mM dextrose. The temperature of the solution was 36 ^oC. Two paladore wires were used to deliver the pulses for direct electrical stimulation. The diaphragm was secured to one of these wires at several points along the rib line. The other electrode was placed around the upper part of the diaphragm, but was not in contact with the muscle. The initial tension after 30-min equilibration period was about 5 g.

The muscle was stimulated directly by subtetanic electrical stimulation. The frequency of stimulation was 14 Hz for 2 s, and series of pulses was applied every 12 s (5 times/min). The isometric contractions were recorded with a microdisplacement myograph transducer (F 50, Narco-Bio-System, Inc; Houston, TX, USA) and recorded on paper (Physiograph IV polygraph). Values for the developed tension (Td) were obtained (for details see Prostran *et al*. 1993).

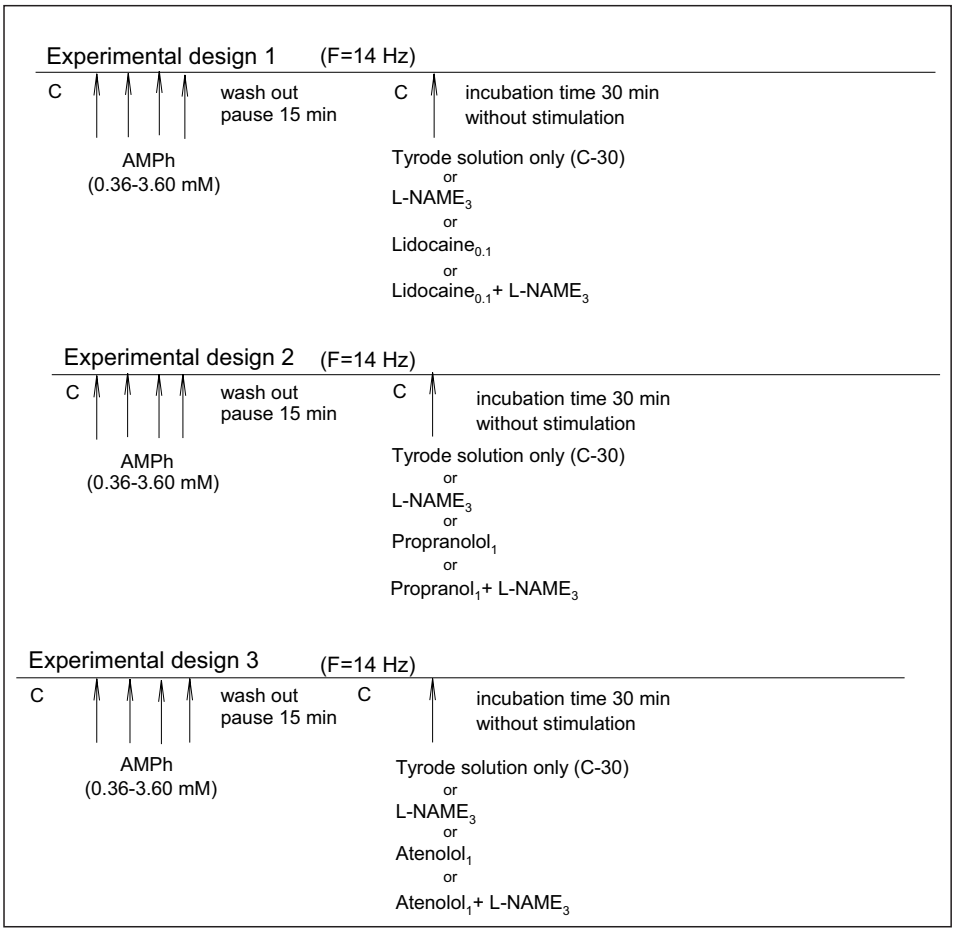

Figure 1. Experimental designs (1-3): C: The value of Td of the electrically stimulated muscle under basal conditions; C-30: The values of Td of the electrically stimulated muscle recorded after 30 min of incubation without drugs, as well as, without electrical stimulation (ES); L-NAME$_3$: the value of Td of the electrically stimulated muscle recorded after 30 min of incubation with L-NAME (3 mM) without ES; Lidocaine$_{0.1}$: the value of Td of the electrically stimulated muscle recorded after 30 min of incubation with lidocaine (0.1 mM) without ES; Lidocaine$_{0.1}$+ L-NAME$_3$: the value of Td of the electrically stimulated muscle recorded after 30 min of incubation with lidocaine (0.1 mM) and L-NAME (3 mM) without ES; Propranolol$_1$: the value of Td of the electrically stimulated muscle recorded after 30 min of incubation with propranolol (1 M) without ES; Propranolol$_1$+ L-NAME$_3$: the value of Td of the electrically stimulated muscle recorded after 30 min of incubation with propranolol (1 M) and L-NAME (3 mM) without ES; Atenolol$_1$: the value of Td of the electrically stimulated muscle recorded after 30 min of incubation with atenolol (1 M) without ES; Atenolol$_1$+ L-NAME$_3$: the value of Td of the electrically stimulated muscle recorded after 30 min of incubation with atenolol (1 M) and L-NAME (3 mM) without ES

The following drugs were used: aminophylline (Aminophyllinum^R, Lek, Ljubljana, Slovenia), N^G-nitro-L-arginine-methyl-ester (L-NAME, Sigma, St. Louis, MO, USA), lidocaine (ICN, Yugoslavia), propranolol (ICN, Yugoslavia), atenolol (ICN, Yugoslavia). Results are expressed as the mean ± S.E.M. for *n* determinations. The difference between the mean values was assessed for significance by Student's *t*-test or one-way analysis of variance (ANOVA followed by *post hoc* 2-sided LSD test), when appropriate. Values of $P<0.05$ were taken as statistically significant (SPSS 8.0 for Windows).

The experimental designs (1-3) are shown in Fig. 1. The effects of the drugs used were expressed as percentage change of Td (%) from the corresponding control.

RESULTS

Isolated rat hemidiaphragm is stimulated by direct subtetanic electrical stimulation (F = 14 Hz). The muscle was pretreated with cumulative concentrations of aminophylline (0.36-3.60 mM) (experimental design 1). Each concentration of aminophylline produced a typical increase in Td of the isolated hemidiaphragm. The effect of each concentration of aminophylline reached its maximum about 5 min after the addition of the drug into the medium. After that, the

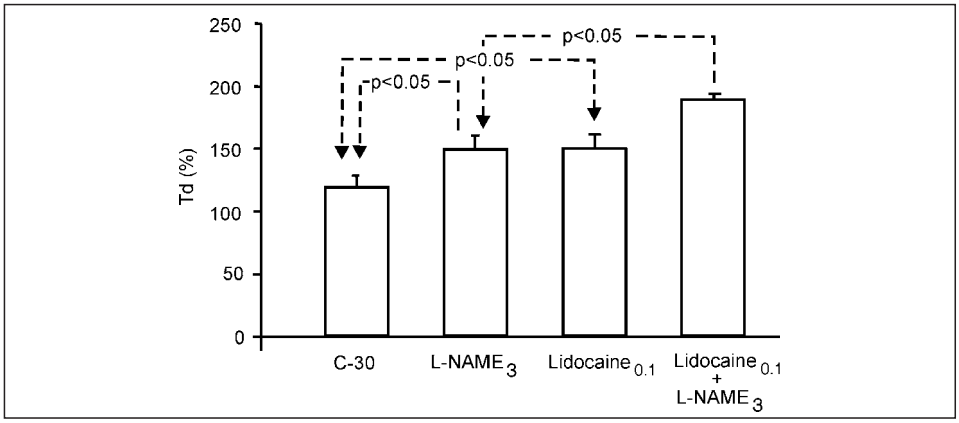

Figure 2. The interaction between lidocaine and L-NAME on Td on the isolated rat hemidiaphragm.
The effects of lidocaine (0.1mM, 30 min of incubation) (Lidocaine$_{0.1}$), L-NAME (3 mM, 30 min of incubation) (L-NAME$_3$) and the combination of lidocaine (0.1 mM, 30 min of incubation) and L-NAME (3 mM, 30 min of incubation) (Lidocaine$_{0.1}$+L-NAME$_3$) on Td during direct subtetanic electrical stimulation; (experimental design 1). C-30: 30 min of incubation of the muscle in Tyrode solution only (without drugs). Each preparation was pretreated with cumulative concentrations of aminophylline (0.36-3.6 mM). Ordinate: percentage change of Td in comparasion with the control values, before the addition of drugs was considered 100%. Each column represents the mean ± S.E.M. (the vertical bars) from 5-6 separate experiments. ANOVA and LSD *post hoc* tests were significantly different $p<0.05$ from the corresponding values

muscle was washed out and rested for 15 min. After a 15 min pause, L-NAME (3 mM, 30 min incubation) or lidocaine (0,1 mM, 30 min incubation) or a combination of both were added into the medium. The effects were recorded after 30 min incubation period (experimental design 1). In a separate series of experiments, the muscle was incubated with the same concentration of lidocaine but only for 5 min. It was found that L-NAME (L-NAME$_3$) significantly ($p<0.05$) increased Td in comparison with the muscle incubated with only the Tyrode solution (C-30) (Fig. 2).

Lidocaine itself (0.1 mM, 5 min incubation) did not change parameters of isometric contractions (not shown). Also, the stimulatory effect of L-NAME on Td (3 mM, 30 min incubation) was preserved after a shorter incubation of the muscle with lidocaine (5 min incubation) (not shown). On the other hand, lidocaine itself (0.1 mM, 30 min incubation) increased Td by approximately 50% ($p<0.05$). The 30 min incubation of the muscle with both lidocaine and L-NAME produced an almost additive stimulatory effect on Td, which increased by 90% (experimental design 1) (Fig. 2).

In a separate series of experiments, muscle was incubated with propranolol (1 μM, 5 or 30 min incubation) or with a combination of L-NAME and propranolol (experimental design 3). It was found that propranolol itself (1 μM, incubation time

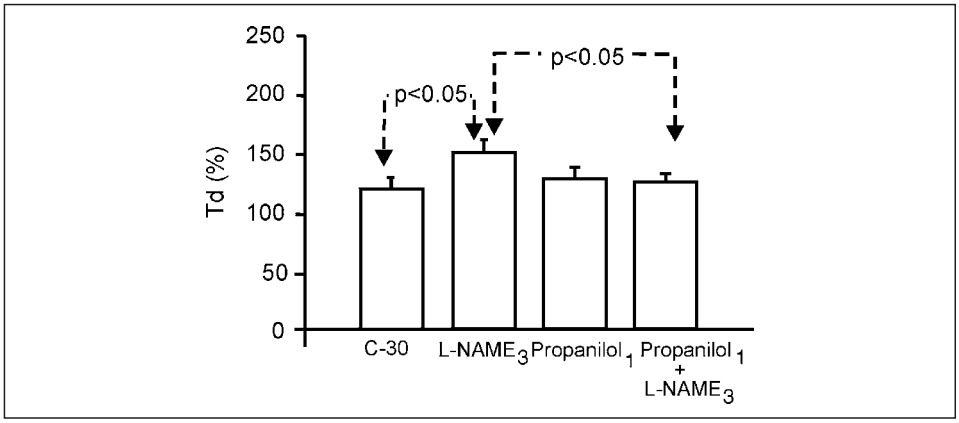

Figure 3. The interaction between propranolol and L-NAME on Td on the isolated rat hemidiaphragm.
The effects of propranolol (1 μM, 30 min of incubation) (Propranolol$_1$), L-NAME (3 mM, 30 min of incubation) (L-NAME$_3$) and the combination of propranolol (1 μM, 30 min of incubation) and L-NAME (3 mM, 30 min of incubation) (Propranolol$_1$+L-NAME$_3$) on Td during direct subtetanic electrical stimulation; (experimental design 2). C-30: 30 min of incubation of the muscle in Tyrode solution only (without drugs). Each preparation was pretreated with cumulative concentrations of aminophylline (0.36-3.6 mM). Ordinate: percentage change of Td in comparasion with the control values, before the addition of drugs was considered as 100%. Each column represents the mean ± S.E.M. (the vertical bars) from 6 separate experiments. ANOVA and LSD *post hoc* tests were significantly different ($p<0.05$) from the corresponding values

5 min) did not change Td. Also, propranolol did not significantly change the L-NAME-induced potentiation of Td (not shown). A longer period of incubation of the muscle with the same concentration of propranolol (1 μM, 30 min incubation) did not change Td but significantly ($p<0.05$) decreased the stimulatory action of L-NAME on Td (Fig. 3).

It was also of interest to assess the effect of selective $beta_1$-blocker (atenolol 1 μM) on L-NAME-induced potentiation of Td. The muscle was incubated with atenolol (1 μM, 30 min incubation) or with a combination of L-NAME + atenolol for 30 min. Atenolol itself (1 μM), did not change Td of the muscle after 30 min of incubation. Also, atenolol did not significantly change the stimulatory effect of L-NAME on Td ($L\text{-}NAME_3$ vs. Atenolol + $L\text{-}NAME_3$, $P>0.05$) (Fig. 4).

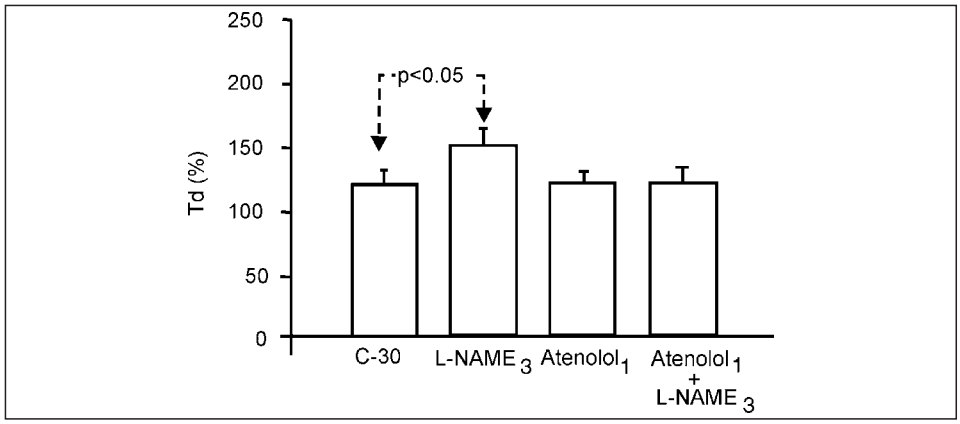

Figure 4. The interaction between atenolol and L-NAME on Td on the isolated rat hemidiaphragm.
The effects of atenolol (1 μM, 30 min of incubation) ($Atenolol_1$), L-NAME (3 mM, 30 min of incubation) ($L\text{-}NAME_3$) and the combination of atenolol (1 μM, 30 min of incubation) and L-NAME (3 mM, 30 min of incubation) ($Atenolol_1 + L\text{-}NAME_3$) on Td during direct subtetanic electrical stimulation (experimental design 3). C-30:30 min of incubation of the muscle in Tyrode solution only (without drugs). Each preparation was pretreated with cumulative concentrations of aminophylline (0.36-3.6 mM). Ordinate: percentage change of Td in comparasion with the control values, before the addition of drugs was considered as 100%. Each column represents the mean ± S.E.M. (the vertical bars) from 4-6 separate experiments. ANOVA and LSD *post hoc* tests were significantly different ($p<0.05$) from the corresponding values

DISCUSSION

We have recently shown (Stojanović *et al*., 2003) that pretreatment of the muscle with cumulative concentrations of aminophylline (0.36-3.60 mM) could be a stimulus strong enough for upregulation of nNOS. Therefore, the observed L-NAME-induced potentiation of diaphragm contractility after cumulative AMPh

pretreatment is most likely due to the specific blockade of nNOS (i.e. the loss of the inhibitory effect of NO on diaphragm contraction).

In the muscle pretreated with cumulative doses of AMPh, lidocaine produced under experimental conditions a significant potentiation of Td. On the other hand, a shorter period of incubation (5 min) of the muscle with the same concentration of lidocaine did not significantly change the muscle contractility. It could be concluded that 30 min incubation is necessary for the pharmacological effect of lidocaine in our experimental model. Obviously, lidocaine could abolish the inhibitory effect of nitric oxide on muscle contractility i.e., the inhibitory effect of NO in the presence of lidocaine was lost. The mechanism(s) by which local anaesthetics produce their effects on muscles has been extensively investigated (Prostran and Varagić, 1981). The primary mechanism of action is the inhibition of membrane voltage-gated sodium channels. Other effects include calcium transport across the sarcoplasmic reticulum. The observed lidocaine-induced potentiation of Td is probably not a consequence of blocked membrane sodium channels as a decrease, instead of an increase of muscle contractility would be expected if such was the case. It is more likely that an interaction with ryanodine receptor is responsible for lidocaine-induced potentiation of Td. Shosan-Barmatz and Zchut (1993) have shown a direct interaction between different local anaesthetics and skeletal muscle ryanodine receptor. They reported that lidocaine increases receptor affinity for ryanodine and the rate of ryanodine association with its binding site. The increase in diaphragm contractility after 30 min incubation with lidocaine is probably due to lidocaine binding and ryanodine receptor activation. Thus, it seems that lidocaine antagonizes the inhibitory action of nitric oxide on muscle contractility. Different effects of lidocaine (5 min vs. 30 min of incubation) on muscle contractility, observed in our present experiments, could be due to pharmacokinetics of the drug in this *in vitro* experimental model.

Also, it was shown that 30 min incubation of muscle with both lidocaine and L-NAME produced almost additive stimulatory effects on Td (Fig. 2). It seems that the observed additive effect is a result of different mechanisms of action of these drugs on muscle contractility: lidocaine *via* interaction with ryanodine receptor, and L-NAME *via* inhibition of nNOS (Martin *et al*., 1993; Mayer and Andrew, 1998).

Propranolol itself (1 μM, incubation time 5 or 30 min), did not significantly change Td of the muscle already pretreated with cumulative concentrations of AMPh (Fig. 3). On the other hand, L-NAME-induced potentiation of Td was antagonized only after 30 min of incubation with propranolol.

It is difficult to assess the exact site of action of propranolol within skeletal muscles. Ha and Fruyer (1997), investigated the effect of propranolol on excitation-contraction coupling in isolated *m. soleus* of the rat *in vitro*. It was shown that propranolol after 30 min of incubation significantly reduced the twitch tension in concentrations above 0.1 μM. At higher concentrations (20 μM), propranolol did not significantly change the amplitude of caffeine induced contractions, suggesting that it did not produce direct inhibition of calcium release from the sarcoplasmic reticulum. On the other hand, direct inhibitory effects of propranolol on calcium uptake by the sarcoplasmic reticulum pump have been shown only at higher concentrations (300-100 μM) (Su and Malencik,

1985) compared to those used in our study. Numerous studies have reported that $beta_2$ adrenergic receptors are predominantly expressed in skeletal muscles in different species (Disatnik *et al*., 1990). Furthermore, Collet *et al*., (1998) have shown that only $beta_2$ receptors are expressed in adult rat diaphragm. We pressume that the observed reduction of the potentiating effect of L-NAME on Td in our experiments may be the result of $beta_2$ adrenergic receptor blockade. This hypothesis is confirmed by the finding that atenolol (1 μM, 30 min incubation), cardioselective $beta_1$ blocker, did not significantly decrease the L-NAME-induced potentiation of Td (atenolol vs. atenolol + L-NAME; $p>0.05$) (Fig. 4). Also, the receptor-independent action of propranolol should not be ruled out. It is possible that a membrane stabilizing action of propranolol could potentially contribute to such an effect.

CONCLUSION

Lidocaine in higher concentrations (0.1 mM) antagonizes the inhibitory effects of nitric oxide on diaphragm contractility. Such interactions occur probably at ryanodine calcium release channels of the muscle, but not at the sarcolemma. In contrast, beta adrenergic blockers antagonize the L-NAME-induced potentiation of Td probably via $beta_2$ adrenergic receptors on the sarcolemma.

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UTICAJ LIDOKAINA I BETA BLOKATORA NA POTENCIRANJE KONTRAKTILNOSTI IZOLOVANE HEMIDIJAFRAGME PACOVA IZAZVANO PRIMENOM L-NAME

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i PROSTRAN MILICA

SADRŽAJ

L-NAME (1-10 mM, 30 min inkubacije) je potencirao tenziju (Td) izolovane hemidijafragme pacova stimulisane direktnom subtetaničkom električnom stimulacijom (DSES) (F=14 Hz). Cilj ovog rada je bio da se ispita moguć modulatorni efekt lokalnog anestetika (lidokain) i beta adrenergičkih blokatora, propranolola i atenolola, na porast izometrijske kontrakcije (IC) izazvane primenom L-NAME. Lidokain (0.1 mM, 5 min inkubacije) nije menjao Td. Takođe, u prisustvu lidokaina (0.1 mM, 5 min inkubacije) stimulatorni efekat L-NAME (3 mM, 30 min inkubacije) je bio očuvan. Nasuprot tome, sam lidokain (0,1 mM 30 min inkubacije) izazvao je porast Td za 50%. Istovremena inkubacija mišića sa lidokainom (0,1 mM 30 min inkubacije) i L-NAME (3 mM, 30 min inkubacije) izazvala je gotovo aditivni stimulatorni efekt na Td za 90%. Inkubacija mišića samo sa propranololom (1 μM, 5 min inkubacije) nije menjala Td. U prisustvu propranolola, L-NAME je zadržala stimulatorni efekt na Td. Sam propranolol (1 μM, 30 min inkubacije) nije menjao Td, ali je poništio stimulatorni efekt L-NAME. Atenolol (1 μM, 30 min inkubacije) nije značajno menjao ni Td niti potencirajući efekt L-NAME na Td.