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RAPID COMMUNICATION

Muscle NMDA Receptors Regulate the Resting Membrane Potential Through NO-Synthase

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Summary

The early postdenervation depolarization of rat diaphragm muscle fibres (8-10 mV) is substantially smaller (3 mV) when muscle strips are bathed with 1 mM L-glutamate (GLU) or N-methyl-D-aspartate (NMDA). The effects of GLU and NMDA are not seen in the presence of aminophosphonovaleric acid (APV), a blocker of NMDA-subtype of glutamate receptors, 5 mM Mg^{2+} (which blocks NMDA-controlled ion channels) and L-nitroarginine methylester (NAME), an inhibitor of NO-synthase. This indicates that NMDA-subtype of GLU receptors might be involved in the regulation of the membrane potential in muscle fibres, most probably through the NO-synthase system.

Key words

Denervation – Resting membrane potential – Neuromuscular junction – NO synthase – NMDA receptor

Introduction

Motor nerves transfer signals to muscle fibre which initiate contractions and coordinate muscle movements. They participate in maintaining muscle fibres in the proper morphological and functional state (Gutmann 1976). The impairment of neurotrophic control by either nerve section or by inhibiting of fast axonal transport produces changes similar to those found following denervation. The earliest change is depolarization of the membrane potential (Albuquerque *et al.* 1971, Shabunova and Vyskočil 1981, Ujec and Dittert 1993) which hinders excitability and contraction. Denervated muscle fibres of the rat diaphragm kept in a tissue culture medium are depolarized by about 8–10 mV (10–12 %) by three hours after denervation (Bray *et al.* 1976, Urazaev *et al.* 1987). At this time the quantal release of acetylcholine (ACh) has not been changed (Nikolsky *et al.* 1985, Zemková *et al.* 1987). The trigger for this early depolarization is not known at present. We report here that this depolarization is substantially smaller in denervated rat diaphragms bathed with 1 mM L-glutamate (GLU) or N-methyl-D-aspartate (NMDA).

For the experiments diaphragms were isolated from male Wistar rats, 180–200 g of body mass under ether anaesthesia. We used 3–5 mm wide strips of parallel intact muscle fibres of the diaphragm with either a phrenic nerve stump 10-20 mm long or without any extramuscular nerve stump (considered as denervated). The muscle strips were pinned with glass needles to the

silicon rubber bottom of transparent glass dishes, with 12 ml of glutamic acid-free No. 199 medium (Hank's salts to which bicarbonate was added to make $NaHCO_3$ = 4.0 g/l, pH 7.2–7.4). Dishes were placed in a moist atmosphere of 5 % CO_2 and 95 % O_2 at 37 °C for 180–200 min. Standard glass microelectrodes (tip resistance 15–20 MΩ, filled with 2.5 M KCl) were used for recording the resting membrane potential (RMP) of 7–20 superficial muscle fibres in the extrasynaptic zone of each strip. Wilcoxon's non-parametric test was used for statistical evaluation of the experiments.

The average RMP of the muscles with the long nerve stump was −74.5±0.4 mV (inside negative, mean±S.E.M, number of fibres n=75) 5 min after dissection and did not change substantially within following 3 h. It was slightly depolarized to −72.0±0.4 mV (n=80, P<0.001) when the muscles were incubated in a medium containing 1 mM GLU for 15 min. GLU (as well as 1 mM NMDA, data not given) thus depolarized the innervated diaphragm muscles by about 2.5 mV.

In the denervated strips (short stump), the average RMP measured within 10–15 min after dissection was −74.5 mV. After 3 h it depolarized by about 8 mV to −66.6 mV (Table 1). When GLU or NMDA was present in the bath, the depolarization was reduced to about 3 mV (to −71.3 or −72.1 mV, respectively, Table 1).

Table 1
Resting membrane potential (in mV, inside negative) of denervated rat muscle fibres after three hours incubation in a culture medium

Control	L-glutamate 1 mM	NMDA 1 mM	L-glutamate 1 mM APV 1mM	L-glutamate 1 mM NAME 0.1 mM
−66.6±0.4 (100)	−71.3±0.3 (75)	−72.1±0.5 (80)	−66.8±0.4 (75)	−65.8±0.5 (90)

L-glutamate 1 mM 5 mM Mg^{2+}	NAME 0.1 mM	L-glutamate 1 mM NAME 0.1 mM L-arginine 1 mM	Nitroprusside 0.1 mM
−66.2±0.5 (75)	−66.3±0.4 (85)	−71.1±0.4 (80)	−70.0±0.4 (65)

NMDA – N-methyl-D-aspartate (Sigma), APV – 2-amino-5-phosphonovaleric acid (Sigma), NAME – L-nitroarginine methylester (Sigma). Numbers of RMP measurements pooled from 5–7 muscles are indicated in brackets. Microelectrode impalements on one muscle strip were made within 5–8 min at 37 °C.

It is known that the effects of GLU are mediated by several types of receptors. One of most common targets is the NMDA receptor which is specifically blocked by APV. If the protection of resting potential by GLU were mediated through NMDA receptors, then block of these receptors should eliminate the GLU effect. Denervated muscles bathed with GLU and the NMDA-receptor inhibitor APV (both in 1 mM concentration) showed the same decrease of the RMP as was observed in the controls (−66.8 mV, see Table 1), i.e. the GLU-evoked protection was completely abolished. Thus the effects of GLU on the early postdenervation depolarization appear to be due to an action on NMDA-type receptors. In several preliminary experiments, non-competitive antagonists MK-801 and CNQX also prevented the GLU-evoked protection of the early postdenervation RMP drop (data not given).

Activation of NMDA receptors opens cation-selective ionic channels which permit Ca^{2+} entry into the cell. In neurones, this influx is inhibited by Mg^{2+} ions

(Jahr and Stevens 1990). In denervated muscles, an increase of Mg^{2+} from 0.3 mM to 5 mM completely eliminated the effect of GLU (Table 1). In other words, the early depolarization is present if the NMDA-receptor-mediated Ca^{2+} entry is inhibited.

In neurones, smooth muscles and other cell types the entry of Ca^{2+} into cytoplasm via NMDA receptors activates NO-synthase, which releases NO molecules from L-arginine. NO can activate the soluble guanylate cyclase which in turn produces cGMP (Baringa 1991, Bredt and Snyder 1992, Vincent and Hope 1992). The cGMP activates specific protein kinases resulting in the final regulatory step – protein phosphorylation. To test whether this cascade is involved in GLU action on postdenervation depolarization, L-nitroarginine methylester (NAME, Sigma), an inhibitor of NO-synthase, was added to the muscle bath together with GLU. At 0.1 mM, NAME completely eliminated the GLU protection of RMP (Table 1). The specificity of its effect was tested by adding the NO-synthase substrate, L-arginine (which competes with NAME for the enzyme, (Leone *et al.* 1994), together with NAME. In a concentration of 1 mM, L-arginine partially reversed the inhibition induced by NAME. This indicates that GLU may maintain the RMP by enhancing NO synthesis. NAME by itself did not influence the postdenervation drop of RMP (Table 1). The effect of nitroprusside, which might serve as a direct donor of the NO group in an aqueous medium (Bugnon *et al.* 1994, O'Drell *et al.* 1991) also speaks in favour of this mechanism. Note that the RMP of denervated muscles kept for three hours in the presence of 0.1 mM sodium nitroprusside was significantly less depolarized (−70 mV) than in the controls (−66 mV, Table 1). Recently, GLU has been found in rat spinal motoneurones and also in the peripheral nerve endings of these neurones by the immunoreactive method (Waerhaug and Ottersen 1993); see also results of Weinreich and Hammerschlag (1975). An mRNA which codes for the amino acid sequence of the GLU transmembrane transporter is present in rabbit motoneurones (Meister *et al.* 1993). The evidence for the presence of GLU in motoneurones and its effect on the postdenervation depolarization of RMP suggests that GLU is released quantally or non-quantally (Antonov and Magazanik 1988) from the motor nerve ending at the neuromuscular junction together or independently of ACh. The GLU triggers the production of NO with subsequent phosphorylation of membrane proteins, channels or transporters involved in RMP maintenance (Forrest *et al.* 1981). Nerve section might impair the GLU release – similarly as has been observed for non-quantal ACh release (Nikolsky *et al.* 1985, Zemková *et al.* 1987) – and subsequent Ca^{2+}-dependent NO production.

Present results are in accord with recent observations that rat skeletal muscles express neuronal-type nitric oxide synthase (Kobzik *et al.* 1994) and that the NO is involved in smooth muscle relaxation and hyperpolarization due to opening of specific type(s) of K^+ channels (Kilpatrick and Cocks 1994, Miyoshi *et al.* 1994). It remains to be ascertained which particular type of channel is influenced by the NO cascade in the membrane of the skeletal muscle.

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