Physiol. Res. 48: 315-317, 1999

RAPID COMMUNICATION

Non-Quantal Acetylcholine Release is Increased After Nitric Oxide Synthase Inhibition

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Received May 10, 1999
Accepted June 14, 1999,

Summary

After anticholinesterase treatment, depolarization of the postsynaptic muscle membrane by about 5 mV develops due to non-quantally released acetylcholine from the motor nerve terminal and can be revealed as hyperpolarization by the addition of curare (H-effect). The H-effect increases significantly to 8.7 mV after inhibition of NO-synthase by L-nitroarginine methylester (L-NAME) whilst no changes in the amplitude and frequency of quantal miniature endplate potentials are observed.

Key words

Acetylcholine • Rat diaphragm • NO synthase • Non-quantal release

Introduction

In addition to the well-established quantal release of acetylcholine (ACh) from nerve terminals, there also exists the so-called non-quantal release (Katz and Miledi 1977, Vyskočil and Illes 1977). There are indications that, at least in rodents, a great part of ACh is released at rest from nerve terminals non-quantally (Mitchell and Silver 1963, Vizi and Vyskočil 1979). While spontaneous quantal release is apparently resistant to the action of nitric oxide (NO) (Lindgren and Laird 1994), no data are available about the effect of this novel neuronal messenger on non-quantal release. The modulation of non-quantal release might be of physiological significance as it has been reported recently that non-quantal ACh can activate the production of NO in muscle fibers, it prevents the early postdenervation depolarization and apparently affects nerve terminals retrogradely (Urazaev *et al.* 1995, 1997, 1998). In the present communication we followed the electrophysiological characteristic of non-quantal release in the rat diaphragm endplate zone before and after inhibition of NO-synthase. In particular, we estimated the size of the so-called H-effect, i.e. the inhibition of

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postsynaptic receptors by tubocurarine, which are depolarized by the ACh released non-quantally from the nerve terminal.

Method

Hemidiaphragms with a 10-15 mm of nerve stump were quickly dissected from female Wistar rats (150-200 g body weight) killed by cervical dislocation and decapitation. The diapragm slices were bathed in a standard oxygenated Krebs-Ringer solution (in mM: NaCl 120.0, KCl 5.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, $NaHCO_3$ 11.0, NaH_2PO_4 1.0, glucose 11.0) enriched with $1x10^{-5}$ mol/l choline chloride at 20 °C to prevent the spontaneous decrease of non-quantal release (Nikolsky *et al.* 1991). The non-quantal release which causes depolarization of muscle fibres in the endplate zone was statistically quantified by impaling, with glass microelectrodes (2.5 mol/l KCl, 10 MΩ) 20 or more fibres during a 5 min period before, and another 20 or more fibres after the addition of $1x10^{-5}$ mol/l (+) tubocurarine (Sigma, USA) to the medium. The difference between the mean resting membrane potentials under these two conditions (H-effect, Vyskočil and Illes 1978, Vyskočil *et al.* 1983) is generally considered to be due to synaptic depolarization which reflects the non-quantal release of ACh. For cholinesterase inhibition, the preparations were treated with an irreversible anticholinesterase, $1x10^{-5}$ mol/l diethoxy-p-nitrophenyl phosphate (armin, Institute of Organic Chemistry, Moscow, Russia) for 30 min and then rinsed several times for 15 min before the measurements were made in a 2 ml Perspex chamber superfused with the saline at a rate 5 ml/min.

Data are expressed as mean ± S.E.M. Parametric analysis of variance (ANOVA) of the experimental groups versus the control group was carried out by multiple comparison using the Bonferroni t-test. Microcal Origin version 3.5 (Microcal Software, Inc. 1991-1994) was used for statistical analyses.

Results and Discussion

Controls

The membrane potential of armin-treated muscles in the endplate zone was –72.2±0.2 mV (number of fibers pooled from 5-7 muscles, n=150). Addition of tubocurarine hyperpolarized the muscle fibers to –77.2±0.2 mV (n=150). The H-effect was thus 5.0±0.2 mV which was considered 100 %.

NO-synthase inhibition

L-NAME (L-nitroarginine methylester, Tocris, USA) in the concentration of $1x10^{-4}$ mol/l was added to the muscle bath during washout of anticholinesterase and then present for the total period of measurements. This treatment increased the membrane potential difference from –72.6±0.3 mV (n=90) before tubocurarine to –81.3±0.3 mV (n=90) after tubocurarine, i.e. H-effect rose by 74 % to 8.7±0.3 mV (n=90, $p<0.05$). When an inactive optic isoform of the inhibitor, D-NAME, was used, the H-effect was not significantly changed (5.2±0.2 mV, n=110, $p>0.05$). This excludes a non-specific action of these arginine esters.

Miniature endplate potentials

The higher H-effect in the presence of L-NAME could be due to an increase of postsynaptic sensitivity to ACh. This can be excluded when amplitudes of uniquantal spontaneous events are unchanged after L-NAME application. Indeed, the mean amplitude and frequency of quantal responses, miniature endplate potentials, did not change during the inhibition of NO synthase by L-NAME; they were 0.87±0.14 mV and 1.5±0.2 events/s in 5 muscle fibres before L-NAME treatment (100 events recorded in each fiber) and 0.90±0.15 mV and 1.6±0.3 events/s after 30 min presence of $1x10^{-4}$ mol/l L-NAME, respectively.

These experiments indicate that NO can modulate the level of non-quantal acetylcholine release at rat neuromuscular junction. If the synthesis of NO is impaired for some reason, the H-effect increases. This increased release can activate the Ca^{2+}-dependent production of NO (Urazaev *et al.* 1997). NO then diffuses from muscle to the nerve terminal and lowers the non-quantal release. Further detailed aspects of this proposed feedback mechanisms are at present studied on muscles *in vivo* and *in vitro* using NO donors and scavengers such as sodium nitroprusside and hemoglobin (Urazaev *et al.* 1996, 1998).

Acknowledgements

We thank Dr. Pavel Hník for careful reading the manuscript and his suggestions. Supported by Grants EU "Nesting", VS 97099, Grant Agency of the Czech Academy of Sciences A7101902 and RBRF 98-04-48044, 99-04-48286 and 99-04-4306.

References

KATZ B, MILEDI R: Transmitter leakage from motor nerve endings. *Proc R Soc London Ser B* **196:** 59-72, 1977.

LINDGREN SA, LAIRD MW: Nitroprusside inhibits neurotransmitter release at the frog neuromuscular junction. *NeuroReport* **5:** 2205-2208, 1994.

MITCHELL JF, SILVER A: The spontaneous release of acetylcholine from the denervated hemidiaphragms of the rat. *J Physiol Lond* **66:** 657-669, 1963.

NIKOLSKY EE, VORONIN VA, VYSKOČIL F: Kinetic differences in the effect of calcium on quantal and non-quantal acetylcholine release at the murine diaphragm. *Neurosci Lett* **123:** 192-194, 1991.

URAZAEV AK, MAGSUMOV ST, POLETAEV GI, NIKOLSKY EE, VYSKOČIL F: Muscle NMDA receptors regulate the resting membrane potential through NO-synthase. *Physiol Res* **44:** 205-208, 1995.

URAZAEV AK, NAUMENKO NV, POLETAEV GI, NIKOLSKY EE, VYSKOČIL F: Nitroprusside decreases the early post-denervation depolarization of diaphragm muscle fibres of the rat. *Eur J Pharmacol* **316:** 219-222, 1996.

URAZAEV AK, NAUMENKO NV, POLETAEV GI, NIKOLSKY EE, VYSKOÈIL F: Acetylcholine and carbachol prevent muscle depolarization in denervated rat diaphragm. *NeuroReport* **8:** 403-406, 1997.

URAZAEV AK, NAUMENKO NV, NIKOLSKY EE, VYSKOČIL F: The effect of imidazole-containing compounds on the postdenervation decrease of membrane potential in the rat diaphragm. *Physiol Res* **47:** 291-295, 1998.

VIZI E.S, VYSKOČIL F: Changes in total and quantal release of acetylcholine in the mouse diaphragm during activation and inhibition of membrane ATPase. *J Physiol Lond* **286:** 1-14, 1979.

VYSKOČIL F, ILLES P: Non-quantal release of transmitter at mouse neuromuscular junction and its dependence on the activity of Na^+-K^+ ATPase. *Pflugers Arch* **370:** 295-297, 1977.

VYSKOČIL F, ILLES P: Eectrophysiological examination of transmitter release in non-quantal form in the mouse diaphragm and the activity of membrane ATPase. *Physiol Bohemoslov* **27:** 449-455, 1978.

VYSKOČIL F, NIKOLSKY E, EDWARDS C: An analysis of the mechanisms underlying the non-quantal release of acetylcholine at the mouse neuromuscular junction. *Neuroscience* **9:** 429-435, 1983.

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