Effects of Carbachol and Adenosine on Neurotransmitter Secretion Induced by Potassium Chloride, Ionomycin, and Sucrose

A. V. Shakirzyanova*, E. E. Nikol'skii*,, and R. A. Giniatullin****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 2, pp. 160-162, February, 2005
Original article submitted August 28, 2004

We compared the effects of adenosine and cholinergic agonist carbachol on spontaneous secretion during local application of K^+, ionomycin, and sucrose increasing Ca^{2+} concentration in the nerve terminal. Adenosine and carbachol had no effect on Ca^{2+} entry, but modulated later stages of exocytosis.

Key Words: *adenosine; carbachol; spontaneous secretion; local application; secretagogues*

Among physiologically active substances capable of modulating quantum secretion of neurotransmitters in the neuromuscular synapse, acetylcholine (ACh) and adenosine (Ade) are of particular interest. They are released from the motor nerve terminal and perform autoregulation of the neurosecretory apparatus [2,5,6]. However, molecular mechanisms of the inhibitory effect of these neurotransmitters remain unclear. Ca^{2+} ions play a key role in quantum secretion of the neurotransmitter from nerve endings.

Here we evaluated whether the inhibitory effect of ACh and Ade on spontaneous quantum secretion of the neurotransmitter is associated with blockade of presynaptic Ca^{2+} channels or these substances modulate neurotransmitter secretion after Ca^{2+} entry into the nerve terminal. To determine the mechanism of action of Ade and nonhydrolyzable ACh analogue carbachol (CCh), we studied the effect of these substances on stimulation of quantum secretion of the neurotransmitter induced by local application of KCl, ionomycin, and sucrose. These compounds increase Ca^{2+} concentration in nerve endings to a different extent and, therefore, activate various components of the secretory apparatus.

*Kazan Institute of Biochemistry and Biophysics, Kazan Research Center of the Russian Academy of Sciences; ** Kazan State Medical University. ***Address for correspondence:*** nastyas@mail.ru. A. V. Shakirzyanova

MATERIALS AND METHODS

Experiments were performed on the preparation of frog sartorius muscle (*Rana ridibunda*). Miniature endplate potentials (MEP) were recorded using glass microelectrodes filled with 2.5 mM KCl (resistance 2-7 MΩ). Cold-blooded vertebrate Ringer's solution containing 113.0 mmol/liter NaCl, 2.5 mmol/liter KCl, 1.8 mmol/liter $CaCl_2$, and 2.5 mmol/liter $NaHCO_3$ was continuously perfused through a bath with the preparation (pH was maintained at 7.2-7.4).

Chemical secretagogues were rapidly and locally applied to the synaptic area. Solutions with increasing concentration of KCl, ionomycin, or sucrose were applied using a glass micropipette with tip diameter of 50-80 μ. Application was performed within 1-2 min after attaining constant MEP under control conditions and during treatment with CCh and Ade.

RESULTS

The frequency of MEP under control conditions was 1.65±0.08 sec^{-1} (n=37). Perfusion of the muscle with Ade (100 μM) and CCh (5 μM) decreased MEP frequency by 62.5±1.5 (n=30, p<0.05) and 35.1±3.1% (n=18, p<0.05), respectively.

Local application of KCl (20 mM), ionomycin (5-15 μM), and sucrose (100 mM) sharply increased MEP

0007-4888/05/1392-0193

frequency, which rapidly reached a constant level. The studied parameters of secretion returned to normal 10-20 min after application. The increase in MEP frequency produced by reapplication of secretagogues (Test 2) was similar to that observed during the 1st application (Test 1, Fig. 1), which allowed comparison of the increase in MEP frequency under control conditions and after application of Ade and CCh on the same synapse.

The effect of Ade and CCh on stimulation of spontaneous secretion produced by application of 20 mM KCl was studied in series I. K^+ activated the same stages of secretion and action potential. K^+ caused depolarization in nerve ending, which activated potential-dependent Ca^{2+} channels, increased Ca^{2+} entry, and initiated the intracellular secretory cascade. KCl less significantly stimulated quantum secretion in the presence of Ade (by 67.0±3.2, n=7, p<0.05) and CCh (by 28.3±3.7, n=6, p<0.05; Fig. 2, *a*). Our results indicate that both substances modulate KCl-induced stage of secretion.

Ionomycin causes rapid Ca^{2+} entry into the terminal without depolarization and activation of Ca^{2+} channels [4]. Ade inhibited the secretory response to ionomycin by 37.9±7.0% (n=4, p<0.05). The increase in MEP frequency after application of ionomycin in the presence of CCh was lower by 23.5±5.2% (n=3, p<0.05; Fig. 2, *b*). Therefore, Ade and CCh had no effect on presynaptic Ca^{2+} channels in frog neuromuscular synapse. These substances modulate the stage that follows Ca^{2+} entry into the terminal.

Sucrose causes Ca^{2+} mobilization from intracellular stores and, therefore, stimulates quantum secretion. Sucrose has no effect on membrane permeability for Ca^{2+}. Ade and CCh inhibited the response to rapid local application of sucrose by 66.5±3.4 (n=5) and 38.5±6.8% (n=4, p<0.05), respectively (Fig. 2, *c*).

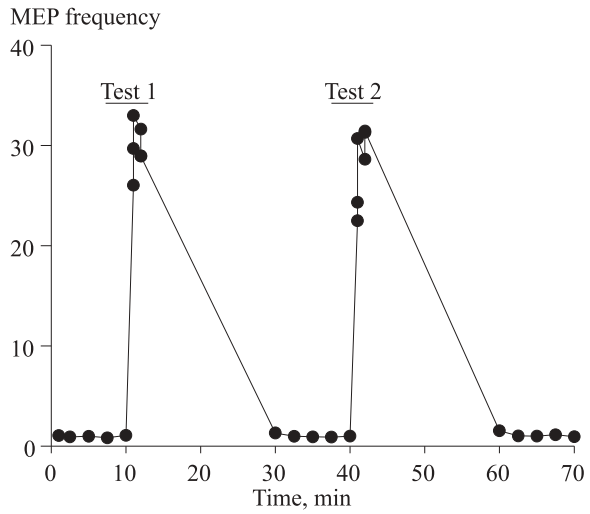

Fig. 1. Frequency of miniature endplate potentials (MEP) after local application of secretagogues.

ACh is released from nerve ending. Ade is formed during hydrolysis of ATP [10]. These substances act as autoinhibitors of neurotransmitter secretion from nerve endings. The mechanism of their effects is poorly understood. The influence of Ade and CCh on Ca^{2+} currents in nerve ending remains unclear [3,7,8,11,12]. Our results indicate that activation of Ade and ACh receptors suppresses quantum secretion of the neurotransmitter, which does not depend on activity of Ca^{2+} channels in frog nerve terminal. Ade and CCh inhibit quantum secretion, but do not modulate Ca^{2+} entry into nerve terminals. They alleviate the effect of intracellular Ca^{2+} mobilized in response to depolarization of the nerve ending. The effect of these neurotransmitters develops at the stages of exocytosis that follow Ca^{2+} entry into the terminal [1]. It cannot be excluded that Ade and CCh affect Ca^{2+}-sequestering systems in the nerve ending. Our previous experiments showed non-

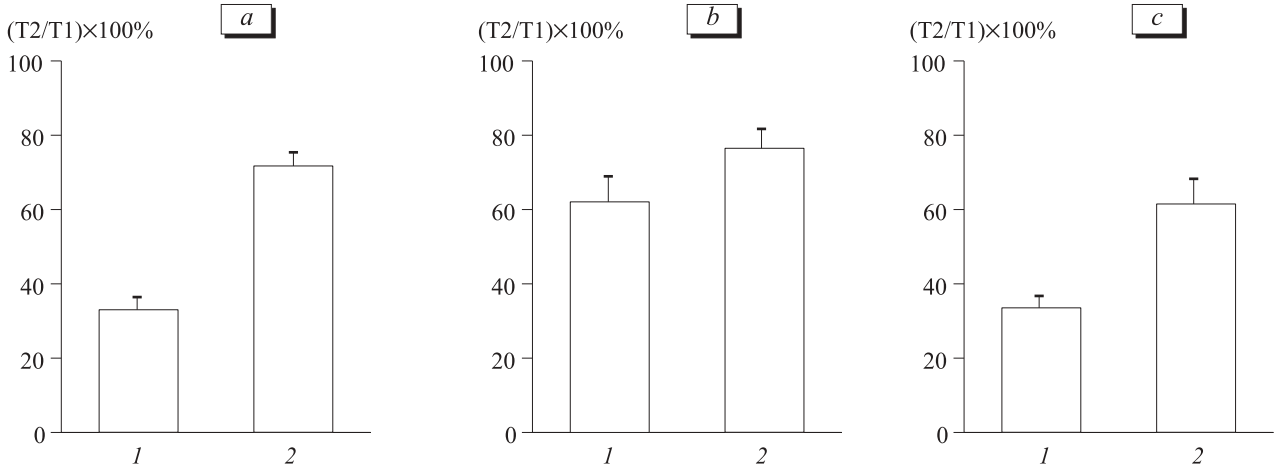

Fig. 2. Increase in MEP frequency after reapplication (Test 2, T2) of 20 mM KCl (*a*), ionomycin (*b*), and sucrose (*c*) in the presence of adenosine (*1*) and carbachol (*2*). Mean frequency of MEP after the first application of secretagogues (Test 1, T1) is taken as 100%.

additive inhibitory effects of Ade and CCh on spontaneous secretion in neuromuscular synapse of frogs [9]. These data suggest that they modulate the same stage of exocytosis.

This work was supported by the Russian Foundation for Basic Research (grant No. 02-04-49994) and NSh-1063.2003.4.

REFERENCES

1. G. J. Augustine, *Curr. Opin. Neurobiol*., **11**, No. 3, 320-326 (2001).
2. B. B. Fredholm, A. P. Ijzerman, K. A. Jacobson, *et al*., *Pharmacol. Rev*., **53**, No. 4, 527-552 (2001).
3. B. R. Hamilton and D. O. Smith, *J. Physiol*., **432**, 327-341 (1991).
4. J. M. Hunt and E. M. Silinsky, *Br. J. Pharmacol*., **110**, 828-832 (1993).
5. E. E. Nikolsky, E. A. Bukharaeva, E. G. Strunsky, and F. Vyskocil, *Ibid*., **104**, 1024-1032 (1991).
6. R. S. Redman and E. M. Silinsky, *Mol. Pharmacol*., **44**, No. 4, 835-840 (1993).
7. R. S. Redman and E. M. Silinsky, *J. Neurosci. Methods*, **57**, 157-159 (1995).
8. R. Robitaille, S. Thomas, and M. P. Charlton, *Can. J. Physiol. Pharmacol*., **77**, 707-714 (1999).
9. A. V. Shakirzyanova and R. A. Giniatullin, *Neurophysiology*, **34**, Nos. 2/3, 237 (2002).
10. E. M. Silinsky and R. S. Redman, *J. Physiol*., **1**, 492, 815-822 (1996).
11. I. Slutsky, H. Parnas, and I. Parnas, *Ibid*., **514**, No. 3, 769-782 (1999).
12. W. Van der Kloot, J. Molgo, and L. A. Naver, *Eur. J. Physiol*., **434**, 735-741 (1997).