CELLULAR MECHANISMS OF INHIBITORY ACTION OF NEUROTRANSMITTERS IN INTESTINAL SMOOTH MUSCLES

I.A. VLADIMIROVA, I.B. FILIPPOV

Bogomoletz Institute of Physiology, Ukraine
phil@biph.kiev.ua

***Iryna Vladimirova**, Doctor of Sciences, Leading Researcher, Department of nerve-muscle physiology, Bogomoletz Institute of Physiology NAS of Ukraine. PhD studentship and thesis under supervision of Prof. P.G. Kostyuk . Field of research interests and area of experience: mediators and membrane mechanisms of the synaptic transmission in the gastro-intestinal smooth muscles and the effect of biologically active substances on neuro-muscular transmission.*

***Ihor Filippov**, PhD in Biology, Senior Research fellow of the Department of Nerve-Muscle Physiology, Bogomoletz Institute of Physiology National Academy of Sciences of Ukraine. Research field and area of experience: biophysics of smooth muscles and mechanisms of intracellular signaling in excitable cells.*

Introduction

Nervous control of the visceral systems plays a significant role in regulation of the motility of the gastrointestinal tract. Inhibitory noncholinergic nonadrenergic synaptic transmission was discovered by a group of Australian scientists in 1963 (Burnstock et al., 1963). Later on, Prof. G. Burnstock suggested that ATP is the mediator of nonadrenergic inhibition (Burnstock et al., 1970). For many years, this neuromuscular transmission could not be suppressed by a variety of well-

known blockers of synaptic transmission despite of its physiological significance. In 1978, Prof. P.G. Kostyuk, interested in our advances and understanding our difficulties in the research work, offered to begin investigations of the action of neurotoxins on synaptic transmission in smooth muscles. Thanks to his great scientific authority, we established fruitful scientific cooperation with the Institute of Bioorganic Chemistry (Moscow, Russia). In common investigations of the action of different neurotoxins, we showed that a polypeptide from bee venom — apamin is more effective in relation to noncholinergic nonadrenergic neuromuscular transmission in gastrointestinal smooth muscles. Thanks to apamin, we succeeded, for the first time, in reversibly blocking nonadrenergic inhibition in smooth muscles. By apamin in low concentrations, inhibitory junction potentials (IJPs) were blocked and unexpectedly in the response to intramural stimulation smooth muscles of caecum guinea pig instead of IJPs were generated noncholinergic nonadrenergic excitatory junction (EJPs) (Vladimirova; Shuba, 1978; Shuba; Vladimirova, 1980). Later on, it was shown that apamin is a selective blocker of low-conductance Ca^{2+}-dependent potassium channels (Banks et al., 1979). These findings led to the extensive use of apamin as an effective and selective tool to probe potassium channels function in different cells.

Apamin-sensitive and apamin-resistant components of inhibitory junction potentials in intestinal smooth muscles

A complex ion and mediator nature of the synaptic transmission in smooth muscles was proved in the experiments with apamin. In particular, it was shown that apamin blocks only fast purinergic component of IJPs and makes visible the other, slow nitrergic, component of these potentials in colonic smooth muscles. The IJPs were totally blocked only under the combined action of the blockers of Ca^{2+}-dependent potassium channels apamin, haloperidol and TEA (Vladimirova; Shuba, 1984; Romanenko at al., 2007). Apamin also blocks the ATP-induced hyperpolarization which supports purinergic hypothesis of nonadrenergic inhibition of intestinal smooth muscles. Studies of inhibitory neuro-muscular transmission in smooth muscles of the human intestine showed that inhibition of the purinergic component of IJPs and of ATP-induced hyperpolarization by apamin is incomplete (Zagorodnyuk et al., 1989). But it is impossible to rule out possibility for the same transmitters to evoke hyperpolarization of the membrane of smooth muscle sells by activation of different Ca^{2+}-dependent potassium channels.

So, apamine-sensitive Ca^{2+}-dependent low-conductance potassium channels and apamin-resistant Ca^{2+}-dependent intermediate-conductance potassium channels can be involved in generation of the purinergic component of IJPs. Ca^{2+}-dependent high-conductance potassium channels participates in the generation of the apamin-resistant nitrergic component of IJPs.

Intracellular mechanisms involved in purinergic inhibition in intestinal smooth muscle

The mechanisms involved in the ATP-induced inhibitory action mediated via metabotropic P2Y receptors and controlling the cell functions are rather complicate (Kugelgen; Wetter, 2000). These receptors are coupled via G_q proteins to phospholipase C and by subsequent increase in $InsP_3$-evoked mobilization of Ca^{2+} from intracellular stores (Marthy; Makhlouf, 1998). Our experiments showed that the amplitude of the fast component of IJPs decreased in the presence of the phospholipase C (PLC) blocker U73122 (Shuba et al. 2003). These findings allowed us to suggest the existence of PLC-dependent and -independent pathways in the mechanisms of IJPs generation. It is necessary to note that noncholinergic EJPs are resistant to the action of U73122. An antagonist of $InsP_3$ receptors, 2-APB (2-aminoethoxyphenyl borate), nearly completely suppressed the purinergic component of IJPs and APT-induced hyperpolarization in intestinal smooth muscles of different regions of the gut. The nitrergic component of IJPs is resistive to the PLC blocker but sensitive to inhibitors of ryanodine receptors of the sarcoplasmic reticulum.

Thus, the above results suppose that local and targeted release of Ca^{2+} from the intracellular Ca^{2+} stores can determine activation of calcium-dependent potassium channels of different conductivities and a final synaptic response in smooth muscles.

Peculiarities of intracellular pathways of synaptic inhibition under conditions of activation of excitatory metabotropic receptors

Investigation of nonadrenergic inhibition in smooth muscles of the intestine usually is performed in the presence of atropine and activation of the P2Y purinoceptors mediated through G-proteins, and the system intracellular messenger evokes Ca^{2+} release from $InsP_3$-sensitive store of the sarcoplasmic reticulum. A question arises: is the same mechanism of the intracellular pathways involved in the condition of activation of excitatory muscarinic receptors ? It is well known that excitatory muscarinic M3 and purinergic inhibitory P2Y receptors are coupled to the same intracellular messenger system. Interaction of muscarinic M3 cholinoceptors with acetylcholine via G_q protein is followed by activation of PLC and by subsequent increase in the level of $InsP_3$; this evokes mobilization of Ca^{2+} from the intracellular store of the sarcoplasmic reticulum. Activation of muscarinic M2 cholinoceptors evokes inhibition of adenylat cyclase via $G_{i/o}$ proteins and activation of nonselective cationic channels. Experiments performed on pre-activated muscarinic receptors of the intestinal smooth muscles showed that the relaxing effect of exogenous ATP was also manifested in the presence of the PLC blocker.

Under these conditions, the inhibitory effect of ATP continued due to activation of $InsP_3$-sensitive receptors because the inhibitory ATP effect was completely blocked after pre-incubation of the preparation together with 2-APB. Note that the amplitude of nonadrenergic IJPs decreased under the influence of 2-APB while that of cholinergic EJPs increased in consequence of this decrease (Vladimirova et al., 2007). Interaction of intracellular signal pathways (probably through G_q protein) was also observed in the case of activation of adenilate cyclase by forskolin under conditions of simultaneous activation of muscarinic cholinoceptors and purionceptors. IJPs were suppressed by forskolin in muscles with no atropine pre-treatment, and cholinergic EJPs appeared instead IJPs (Philyppov et al., 2004). However, in atropine pre-treated smooth muscles, forskolin exerted no influence on nonadrenergic IJPs. This effect of forskolin is not due to the increase of the intracellular level of cAMP as cell-permeable cAMP analog dibutyryl cAMP did not cause significant changes in IJPs.

Under the conditions of selective pre-activation of M2 or M3 muscarinic cholinoceptors, the mechanism of intracellular signaling mediating of inhibition was modified. The $InsP_3$-dependent pathway which determines purinergic inhibition of smooth muscles is switched off, and the inhibitory action of neurotransmitters is realized under such conditions through the $InsP_3$-independent pathway (Vladimirova et al., 2007). The above-described results put changes in current opinions about stability of transduction of intercellular signal pathways by activation of varius types of receptors.

Acknowledgments. We are very thankful to Prof. P.G. Kostyuk for his attention to our investigation and fruitful discussions of our results.

REFERENCES

Banks BE, Brown C, Burgess GM, Burnstock G, Claret M, Cocks TM, Jenkinson DH, 1979. Apamin blocks certain neurotransmitter-induced increases in potassium permeability. Nature 22: 415-417.

Burnstock G, Campell G, Bennett M, Hoplman M, 1963.Inhibition of smooth muscle of the taenia coli. Nature 200: 581-583.

Burnstock G, Campbell G, Satchell D, Smythe A, 1970. Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. Br. J. Pharmacol 40: 668-88.

Filyppov IB, Vladimirova IA, Ganitkevich V, Ya, Shuba MF, 2004. Adenylate cyclase-mediated modulation of interaction between excitatory and inhibitory synaptic influences on smooth muscles. Neurophysiology 36: 438-445.

Kugelgen I, Wetter A, 2000. Molecular pharmacology of P2Y-receptors. Naunyn-Schmiedeberg's Arch. Pharmacol 362: 310-323.

Marthy K, Makhlouf G, 1998. Coexpression of Ligand-gated P2X and G Protein-coupled P2Y Receptors in Smooth muscle. J. Biol. Chem 273: 4695-4704.

Romanenko A, Grusha M, 2007. Effects of haloperidol and clotrimazole on synaptic transmission and contractile activity of smooth muscles of the guinea-pig intestine. Neurophysiology. 39: 412-415.

Shuba M, Vladimirova I, 1980. Effect of apamin on the electrical responses of smooth muscles to adenosine 5-triphosphate and to non-adrenergic, non-cholinergic stimulation. J. Neuroscience 5: 583-589.

Vladimirova IA, Shuba MF, 1978.The effect of strychnine, hydrastine and apamin on synaptic transmission in smooth muscle cells. Neurophysiology 10: 295-299.

Vladimirova IA, Shuba MF, 1978. Synaptic processes in smooth muscles. Neurophysiology 9: 307-319.

Vladimirova IA, Filippov IB, Kulieva EM, Dyskina YuB. Ganitkevich VYa, 2007. Differences between cellular mechanisms of ATP-and noradrenaline-induced inhibition of visceral smooth muscles under conditions of selective and combined activation of M2 or M3 cholinoreceptors. Neurophysiology 39: 20-29.

Zagorodnyuk VP, Vladimirova IA, Vovk EV, Shuba MF, 1989. Studies of the inhibitory non-adrenergic neuromuscular transmission in the smooth muscle of the normal human intestine and from a case of Hirschsprung's disease. J. Auton. Nerv. Syst 26: 51-60.