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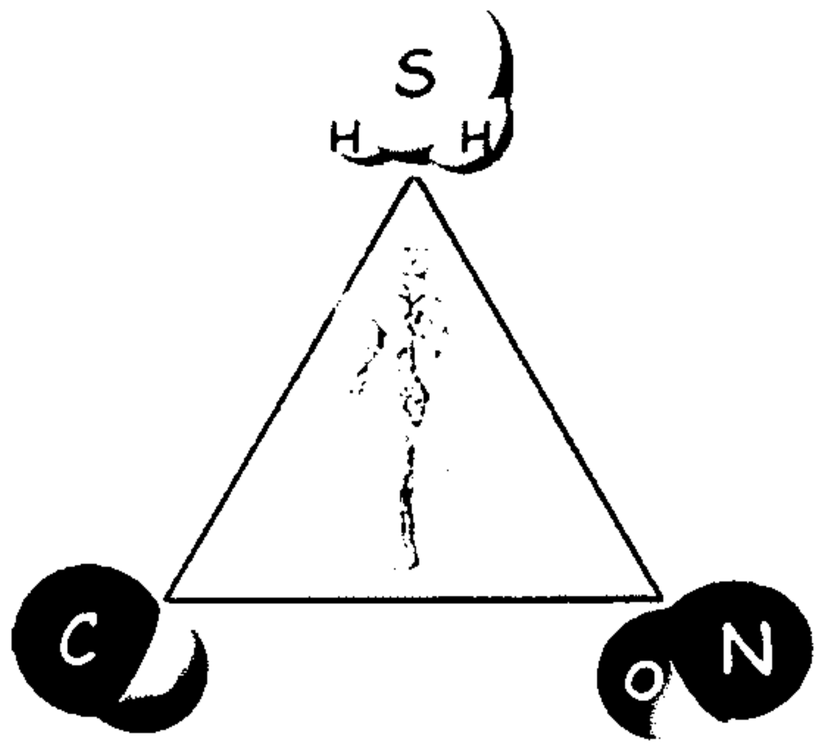

Gasotransmitters : Physiology and Pathophysiology

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We also have studied the effects of short-term applications of the β2-ARs agonist on the contractility and nitric oxide production. After addition of 5 μM fenoterol, a delayed increase in the amplitude of atrial contractions was recorded after removal of the agonist from the medium; the dynamics and intensity of the inotropic reaction depended on the duration of fenoterol application. During short-term agonist applications for 1 or 3 min, no significant changes were detected in the strength of contraction; however, 15 min later after agonist removal from the outer solution, the amplitude of contractions increased and reached 116±4 (n=6, p< 0.01) and 140 ± 3.5% (n=6, p<0.01) respectively. Against the background of the effect of a selective beta-2-AR blocking agent (0.1 μM ICI-118.551), there were no delayed positive inotropic effects of fenoterol. NO production increased in atrial cardiomyocytes against the background of fenoterol effect. A short 1-min application led to a significant increase in DAF-FM fluorescence up to 102.1±0.3% (n=6, p<0.05). However, after removal of the agonist, the level of fluorescence reduced down to 98.6 ± 0.4% for 5 min (n=6, p< 0.05). The 3-min fenoterol application led to an increase in fluorescence up to 103.5 ±0.4% (n=6, p<0.05); after removal of the agonist, the level of fluorescence decreased rapidly to 92.6±0.7% for 5 min (n=6, p<0.05).

Thus, the dynamics of NO production regulates the positive inotropic response induced by the long and short-term fenoterol application. In these cases the NO acts as factor (clamp) which prevents increase of contractility during activation of β2ARs.

This study was supported by Russian Foundation for Basic Research (project no. 14-04-00312-a) and MK-108.2013.4.

EFFECTIVENESS AND SAFETY OF GASOMEDIATORS IN CLINICAL PRACTICE

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Experience of practical use of ozone as a drug in different medicine directions is strongly shown the possibilities of reactive oxygen species for sanogenic aims. There are singlet oxygen, nitric oxide, carbon monoxide, is being a gasomediators. Modern technologies is using in Russian clinics allow to use the bioregulatory properties of endogenous gasomediators by its modulation with different drugs or physical factors in vivo. These methods may be used at different pathology by transport of bioregulators in gaseous, soluted in biological fluid or in the prodrug composition. For this task the special technical devices is producing indicated bioregulators and new drugs are developed.

At urgent pathology with hypoxia the general organism reaction includes disorders of pro- and antioxidant balance is known as one of the main pathogenic mechanisms for different diseases. Most marked result of this dismetabolism variants is the oxidative stress, which is producing with participation of primary radicals of oxygen, lipids, nitrogen and chlorine; its derivates – reactive molecules (singlet oxygen, hydrogen peroxide, lipid peroxides, peroxynitrite, hypochlorite etc.) and secondary radicals (hydroxyl radical and lipid radicals). It is clear, that at urgent state the use of any biological radicals for sanogenic aims

determine the necessary of the control of its level in vivo and biological response on the administration of reactive oxygen species.

On the ozone therapy example we designed a new technology of the individual selection of optimal ozone doses and the antioxidant choice for each patient. This technology is based on the use of blood biochemiluminescense analysis. Our method is realized on Russian biochemiluminometer BHL-10 (Nizhny Novgorod, Russia) with real-time control of pro- and antioxidant states for the reactive oxygen species administration and timely correction of the gasomediator therapy.

Our experimental and clinical investigations for proposed technology use (on the ozone therapy example) in correction of burn disease-associated hypoxia confirm its effectiveness, which based on the gasomediators sanogenic activity.

NITRIC OXIDE REGULATES AN ACETYLCHOLINESTERASE ACTIVITY IN THE MAMMALIAN NEUROMUSCULAR JUNCTION

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Acetylcholinesterase (AChE) is an enzyme which determines predominantly the lifetime of acetylcholine in cholinergic synapses (Katz and Miledi, 1973). AChE density in the synaptic cleft is not constant and can adapt to the changes in the synaptic activity. The rate of AChE synthesis and, therefore, the amount of the enzyme, may depend on the pattern of the firing (Pregelj et al., 2007). However all known mechanisms of AChE regulation require dozens of hours, since synthesis, secretion and anchoring of the enzyme are required (for rev. see Rotundo *et al.*, 2003; 2008). Taking into account that ambient changes and, therefore, the modulation of the pattern of physiological activity, can happen rapidly, the existence of some faster pathways to regulate AChE activity (activation or inhibition) might be proposed. Considering a partial AChE inhibition as a highly effective way to modulate the amplitude of synaptic potentials (what widely used in medical practice), one can imagine that a fine tuning of AChE activity by endogenous factors may take place as well, for example to underlie the synaptic plasticity. Until recently, however, practically nothing was known about the existence of endogenous modulators of AChE activity.

We demonstrated the existence of an endogenous mechanism of regulation of the synaptic AChE activity. We showed that at the rat *extensor digitorum longus* neuromuscular junction an activation of N-methyl-D-aspartate (NMDA) receptors by a combined application of glutamate and glycine leads to the enhancement of nitric oxide (NO) production resulting in partial AChE inhibition. The latter reveals itself as an increase in the miniature endplate current amplitude. The inhibition of AChE by paraoxon, the inactivation of NO synthase by

N^{ω}-nitro-L-arginine methyl ester, as well as the blockade of NMDA receptors by DL-2-amino-5-phosphopentanoic acid prevent the increase in the miniature endplate current amplitude caused by amino acids. Motor nerve stimulation with a frequency of 10 Hz in glycine containing bathing solution also resulted in an increase in the amplitude of miniature endplate currents recorded in the interstimuli intervals. Preliminary inhibition of NO synthase as well as blockade of NMDA receptors fully eliminated this effect. This suggests that endogenous glutamate, released into the synaptic cleft as a co-mediator of acetylcholine, is capable of triggering the NMDA receptor/NO synthase mediated pathway of modulation of the synaptic AChE activity.

We conclude that in addition to well-established modes of the synaptic plasticity, including changes in the effectiveness of the neurotransmitter release and/or the sensitivity of postsynaptic membrane, one more, previously unknown mechanism exists based on the prompt regulation of the timing of acetylcholine action in the synaptic cleft. Assuming that the partial AChE inhibition is a very effective way to elevate the amplitude of synaptic responses one can imagine the possibility of down regulation of this enzyme activity by endogenous substances to compensate for the decreased quantal content (e.g. during synaptic fatigue) or reduced sensitivity of postsynaptic membrane (at different pathologies). To our opinion, the down regulation of AChE activity may not only compensate for the pathological reduction of a safety factor but serve as a regulatory mechanism.

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EFFECT OF SODIUM NITROPRUSSIDE ON ELECTROPHYSIOLOGICAL PROPERTIES OF THE LARGE INTESTINE SMOOTH MUSCLES ON THE BACKGROUND OF INTRACELLULAR PH CHANGES

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Studies of the nitric oxide effects in a changing intracellular pH on smooth muscle cells (SMC) of the intestine, due to a high interest in this matter, as a possible mediator .

Electrical parameters and contractile activity of SMC circular layer of the proximal large intestine (PLI) of cats within 1.0 - 1.5 cm from Bauginovoy flap were by the double simultaneously recorded "bridge sucrose".

In normal Krebs solution the circular SMC PLI had no the original spontaneous electrical and contractile activity. Hyperpolarizing pulses of electric current led to the development of an anelectrotonus potential. Depolarizing pulses of electric current led to the emergence of katelectrotonus potential (KEP), with generation at its plateau of the 2-3 action potentials and the simultaneous development of the phasic contractile responses.

Sodium nitroprusside (HNa) (at a concentration of 10-4 M) caused the initial hyperpolarization of the membrane potential (MP), the reduction of the membrane resistance by 46,2 ± 2,29% (n = 12 ; p <0.05) and induced contractile responses by 71 7 ± 2,84% (n = 12 ; p <0.05), compared to control values in normal Krebs solution .

At a concentration of 10^{-2} M tetraethylammonium (TEA) caused a slight increase in the membrane resistance, the emergence of anodobrekeal electrical and contractile response. 4-8 AP were recorded at the plateau of KEP with simultaneously increase of the induced contractile response.

On the background of TEA, HNa (10-4 M) did not cause any change in the membrane potential. Membrane resistance was reduced by 19,6 ± 0,86% (n = 7 ; p < 0.05), PO disappeared throughout the action of sodium nitroprusside . Magnitude of the contractile response was 66,2 ± 3,26% (n = 7 ; p < 0.05) from baseline values. Closing effects of HNa characterized by a slight increase in tone and a restore the original settings after 8 - 13 minutes.

A standard technique with NH_4Cl for changes in intracellular pH was used. NH_4Cl ($2x\ 10^{-2}M$) caused a reduction in the resistance of the membrane and the induced contractile responses, compared to baseline values . Expiry of NH_4Cl effect characterized by a slight increase in muscle tone, reduction in the membrane resistance and increasing in an amplitude the membrane of response contraction, compared to control values in Krebs solution .

On the background of NH_4Cl, sodium nitroprusside (10^{-4} M) had no effect on the value of the MP. Decrease in the membrane resistance was 50,25 ± 1,84% (n = 8 ; p < 0.05) and contractile responses was fully suppressed. Expiry of NH_4Cl characterized by increased tone, partial recovery of resistance to 73,7 ± 3,05% (n = 8 ; p < 0.05) and induced contractile responses (26,9 ± 1,16%; n=8 ; p < 0.05), compared to control values in Krebs solution . These effects of HNa leveled against the backdrop of TEA.

Thus, during intracellular alkalization the increase in the inhibitory effect of sodium nitroprusside on the electrophysiological parameters was observed, in contrast to the intracellular acidification. These effects is partly due to the membrane potassium conductivity of the SMC PLI.

NEUROCHEMICAL SIGNALIZATION IN PROLIFERATING AND DEFERENTIATING CELLS OF PRIMARY CULTURE IN THE BRAIN OF TROUT ONCORHYNCHUS MIKISS

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After injury of the central nervous system, in particular the spinal cord, the locomotor activity of the fish can effectively recover. It concerns both the ability of the central projection neurons to regenerate damaged axons, and the emergence of new cells in the zone of injury, and the occurrence of high proliferative activity in nearby neurogenic niches and proliferative areas of the brain. Mechanisms of the such high reparative activity in the nervous tissue of fish, including both anatomic and functional regeneration, remains unexplored. After applying a mechanical injury to the eye by the damage to the structure of