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MECHANISMS OF HYDROGEN SULFIDE (H_2S) ACTION ON SYNAPTIC TRANSMISSION AT THE MOUSE NEUROMUSCULAR JUNCTION

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Abstract—Hydrogen sulfide (H_2S) is a widespread gasotransmitter also known as a powerful neuroprotective agent in the central nervous system. However, the action of H_2S in peripheral synapses is much less studied. In the current project we studied the modulatory effects of the H_2S donor sodium hydrosulfide (NaHS) on synaptic transmission in the mouse neuromuscular junction using microelectrode technique. Using focal recordings of presynaptic response and evoked transmitter release we have shown that NaHS (300 μM) increased evoked end-plate currents (EPCs) without changes of presynaptic waveforms which indicated the absence of NaHS effects on sodium and potassium currents of motor nerve endings. Using intracellular recordings it was shown that NaHS increased the frequency of miniature end-plate potentials (MEPPs) without changing their amplitudes indicating a pure presynaptic effect. Furthermore, NaHS increased the amplitude of end-plate potentials (EPPs) without influencing the resting membrane potential of muscle fibers. L-cysteine, a substrate of H_2S synthesis induced, similar to NaHS, an increase of EPC amplitudes whereas inhibitors of H_2S synthesis (β-cyano-L-alanine and aminooxyacetic acid) had the opposite effect. Inhibition of adenylate cyclase using MDL 12,330A hydrochloride (MDL 12,330A) or elevation of cAMP level with 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate (pCPT-cAMP) completely prevented the facilitatory action of NaHS indicating involvement of the cAMP signaling cascade. The facilitatory effect of NaHS was significantly diminished when intracellular calcium (Ca^{2+}) was buffered by 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis acetoxymethyl ester (BAPTA-AM) and ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid acetoxymethyl ester (EGTA-AM). Activation of ryanodine receptors by caffeine or ryanodine increased acetylcholine release and prevented further action of NaHS on transmitter release, likely due to an occlusion effect. Inhibition of ryanodine receptors by ryanodine or dantrolene also reduced the action of NaHS on EPC amplitudes. Our results indicate that in mammalian neuromuscular synapses endogenously produced H_2S increases spontaneously and evoked quantal transmitter release from motor nerve endings without changing the response of nerve endings. The presynaptic effect of H_2S appears mediated by intracellular Ca^{2+} and cAMP signaling and involves presynaptic ryanodine receptors.

Key words: hydrogen sulfide, neuromuscular junction, transmitter release, L-cysteine, adenylate cyclase, ryanodine receptors.

INTRODUCTION

Hydrogen sulfide (H_2S), is a gaseous transmitter along with nitric oxide (NO) and carbon monoxide (CO) (Kimura, 2010, 2011; Hermann et al., 2012; Paul and Snyder, 2012; Wang, 2012, 2014). Several important biological actions of H_2S have been identified including regulation of blood pressure, insulin release, cytoprotection, smooth muscle relaxation and neuronal excitability (Hosoki et al., 1997; Kimura and Kimura, 2004; Kawabata et al., 2007; Sitdikova et al., 2010; Hermann et al., 2012; Wang, 2012, 2014; Kuksis et al., 2014). Endogenous production of H_2S in mammalian tissues occurs mainly through three enzymes: cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase along with an additional contribution of cysteine aminotransferase (Abe and Kimura, 1996; Kamoun, 2004; Shibuya et al., 2009; Kimura, 2011, 2014). Ion channels are a main target of

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Abbreviations: AOAA, aminooxyacetic acid; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N′,N′-tetraacetic acid tetrakis acetoxymethyl ester; CBS, cystathionine β-synthase; CO, carbon monoxide; CSE, cystathionine γ-lyase; EGTA-AM, ethylene glycol-bis(2-aminoethyle ther)-N,N,N′,N′-tetraacetic acid acetoxymethyl ester; EPCs, end-plate currents; EPPs, end-plate potentials; H_2S, hydrogen sulfide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MDL 12330A, MDL 12,330A hydrochloride; MEPCs, miniature end-plate currents; MEPPs, miniature end-plate potentials; NaHS, sodium hydrosulfide; NO, nitric oxide; pCPT-cAMP, 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate; RyR, ryanodine receptors; β-CA, β-cyano-l-alanine.

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