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Research report

Hydrogen sulfide depolarizes neurons in the nucleus of the solitary tract of the rat

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ABSTRACT

Hydrogen sulfide (H_2S) is a gasotransmitter that has been described to affect the membrane potential of neurons in a number of brain areas. Using whole cell patch-clamp electrophysiological techniques, we investigated the effects of H_2S on the membrane potential of neurons in the nucleus of the solitary tract (NTS). Whole cell patch clamp recordings were obtained from 300 μm coronal NTS brain slices and bath application of the H_2S donor, sodium hydrosulfide (NaHS)(1 mM, 5 mM and 10 mM) was shown to have clear concentration-dependent, reversible, depolarizing effects on the membrane potential of 95% of neurons tested (72/76), an effect which in 64% (46/72) of these responding neurons was followed by a hyperpolarization. In the presence of the voltage-gated sodium channel blocker tetrodotoxin (TTX) and the glutamate receptor antagonist kynurenic acid (KA), these depolarizing effects of 5 mM NaHS (5.0±2.2 mV (n=7)) were still observed, although they were significantly reduced compared to regular aCSF (7.7±2.0 mV (n=7), p^*<0.05, paired t-test). We also demonstrated that hyperpolarizations in response to 5 mM NaHS resulted from modulation of the K_{ATP} channel with recordings showing that following K_{ATP} channel block with glibenclamide these hyperpolarizing effects were abolished (Control −7.9±1.2 mV, Glibenclamide −1.9±0.9 mV (n=8) p<0.05, paired t-test). This study has for the first time described post-synaptic effects of this gasotransmitter on the membrane potential of NTS neurons and thus implicates this transmitter in regulating the diverse autonomic systems controlled by the NTS.

1. Introduction

Hydrogen sulfide (H_2S) is a toxic gas which has recently been found to have an endogenous role in the gastrointestinal system (Fiorucci et al., 2005; Strege et al., 2011), the vasculature (Al-Magableh and Hart, 2011; Yang et al., 2008; Zhao et al., 2001), and the central nervous system (CNS) (Abe and Kimura, 1996). Endogenous H_2S can be produced by three different pathways in the body as a result of the actions of cysthathionine γ-lyase (CSE) (Stipanuk and Beck, 1982), cysthathionine β-synthase (CBS) (Abe and Kimura, 1996), or a combination of cysteine aminotransferase (CAT) and 3-mercaptopyruvate (3-MST) (Shibuya et al., 2009). Different tissues preferentially use specific pathways to produce H_2S, with both the CBS and 3-MST pathways being the significant H_2S producing pathways within the CNS (Abe and Kimura, 1996; Shibuya et al., 2009). These enzymes

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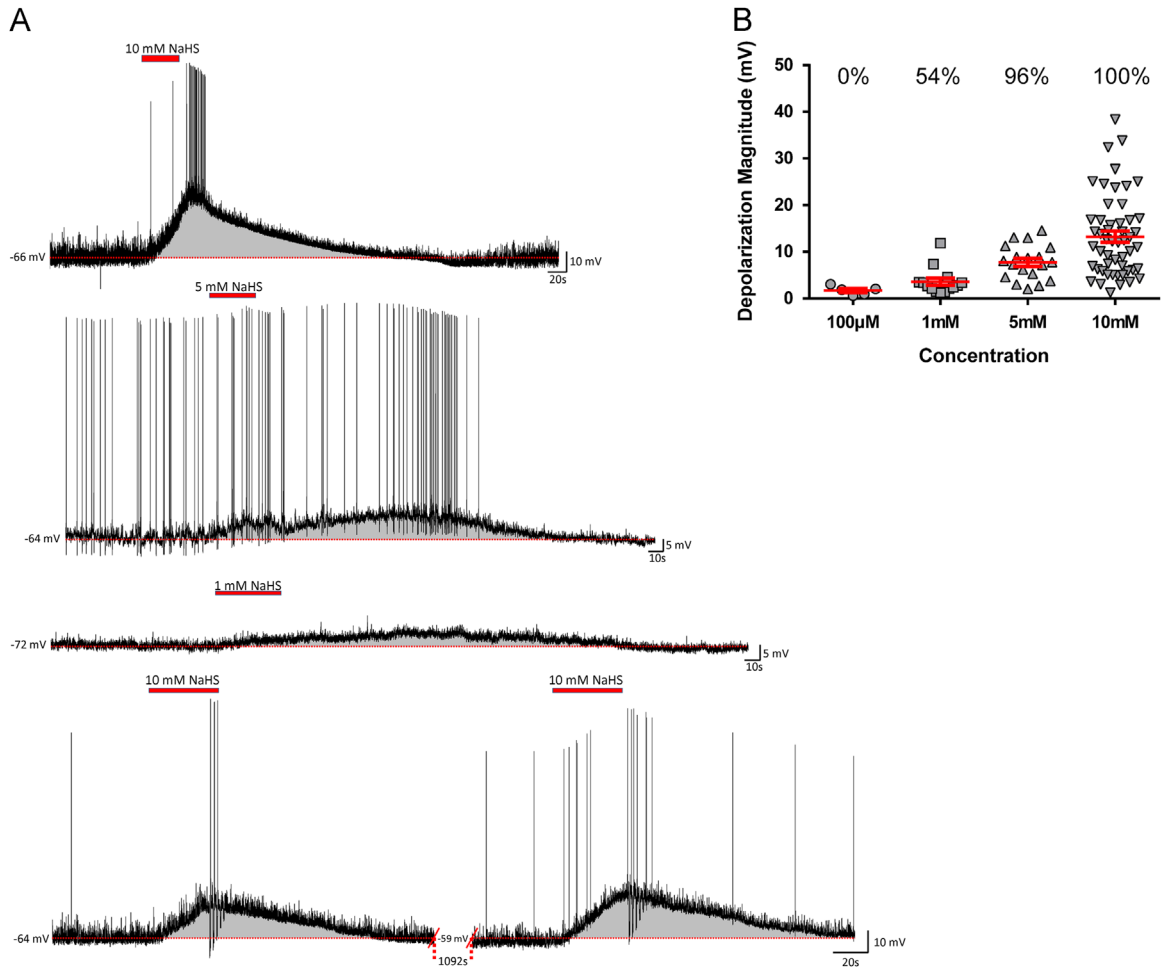

Fig. 1 – NTS neurons depolarize in response to NaHS. A. Current-clamp recordings from NTS neurons showing the effects of 10 mM, 5 mM and 1 mM NaHS (period of administration indicated by the red bars) on membrane potential. Notice the rapid depolarizing effects of NaHS which are followed by return to baseline membrane potential. B. This scatterplot shows the depolarization magnitude for every neuron tested at four different concentrations of NaHS, while the black lines and error bars represent the mean ± SEM for each individual group. Percentage of responders at each concentration are indicated at the top of the graph. C. A current-clamp recording from a NTS neuron showing the repeatability of the effects of NaHS within a single neuron. Hyperpolarizing current steps were applied in 10 pA increments from −50 pA to −10 pA at the peak of both responses.

have been shown to be present in specific regions of the CNS including the paraventricular nucleus (PVN) (Streeter et al., 2011), subfornical organ (SFO) (Kuksis et al., 2014), nucleus tractus solitarius (NTS) (Austgen et al., 2011), hippocampus and cerebellum (Abe and Kimura, 1996). These areas play important roles in the regulation of autonomic function, memory formation and motor function. In addition H_2S has been shown to influence cardiovascular function in the NTS (Qiao et al., 2011), the hypothalamus (Dawe et al., 2008) and the lateral cerebral ventricle (Ufnal and Sikora, 2011;Ufnal et al., 2008), as well as respiratory function in the hypoglossal rootlets (Hu et al., 2008). At the cellular level H_2S facilitates long term potentiation in the hippocampus (Abe and Kimura, 1996), and influences membrane potential of neurons in the PVN (Khademullah and Ferguson, 2013), the dorsal raphe nucleus (Kombian et al., 1993) and SFO (Kuksis et al., 2014).

The NTS is an area located in the dorsomedial medulla adjacent to the area postrema and the dorsal motor nucleus of the vagus. It plays critical roles in the central regulation of cardiovascular (Doba and Reis, 1973), respiratory (Furuya et al., 2014) and metabolic functions (Dallaporta et al., 1999). The NTS is an important site of integration of afferents from the viscera, such as the heart, lungs and gastrointestinal tract (Kalia and Mesulam, 1980), as well as afferents from higher brain nuclei including the PVN, arcuate nucleus, and the central nucleus of the amygdala(van der Kooy et al., 1984). Recently microinjection of NaHS into the NTS has been shown to decrease heart rate and blood pressure (Qiao et al., 2011). Furthermore, *in vitro* studies recording from NTS neurons have shown that H_2S increases solitary tract evoked post-synaptic currents through facilitated presynaptic glutamate release. However, this study reported no effects on the resting membrane potential of NTS neurons (Austgen et al., 2011).

Post synaptic effects of H_2S on the membrane potential, and thus excitability of CNS neurons, have been reported in dorsal raphe (Kombian et al., 1993), PVN (Khademullah and Ferguson, 2013), SFO (Kuksis et al., 2014), and dorsal root ganglion neurons (Andersson et al., 2012). The effects in the