Published in final edited form as:

Acta Physiol (Oxf). 2014 February ; 210(2): 231–232. doi:10.1111/apha.12204.

ANTEROVENTRAL PREOPTIC NEURONS, CONTROL OF BREATHING AND H₂S: PHYSIOLOGY OR TOXICOLOGY?

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Dear Sir,

We have read with great interest the recent study from Kwiatkoski et al. (Kwiatkoski et al., 2013), in which microinjections of Na₂S were performed either in the 3rd ventricle or directly into the anteroventral preoptic region of the rat. Following these injections, the ventilatory response to hypoxia was found to be reduced.

This observation suggested to the authors that specific populations of neurons in the anteroventral preoptic region could be part of a neuronal network modulating the response to hypoxia via changes in local endogenous H₂S concentrations. However, inferring a role for endogenous hydrogen sulfide as the physiological mediator of this response, from the use of exogenous H₂S must first solve a quite challenging conundrum. Indeed, while the 260 nmol of H₂S that were injected into the third ventricle may seem like a “small” quantity, the dilution of this amount of sulfide in 2 μ l (the volume used for injection) corresponds to a concentration of 130 millimolar (mM). The dreadful toxicity of H₂S relates to its ability to combine immediately with the cytochrome c oxidase and stop ATP production, akin to the effects of cyanide (Smith and Gosselin, 1976). In intact cells, mitochondrial respiration is inhibited at concentrations of H₂S ranging from 10–30 micromolar (μ M) (Cooper and Brown, 2008, Lagoutte et al., 2010). This inhibition may even start at much lower concentrations (Modis et al., 2013). In vivo, signs of H₂S toxicity for the medullary neurons are observed for concentrations of diffusible gaseous sulfide in the blood of few μ M in the rat (Klingerman et al., 2013), while concentrations lower than one μ M are able to stimulate the arterial chemoreceptors (Klingerman et al., 2013). These figures must therefore be compared to the 100 mM of H₂S, i.e. a concentration several orders of magnitude higher than that required to produce a cytotoxic anoxia, which were applied onto populations of neurons close to the third ventricle. The neurons directly exposed to these solutions must in fact have experienced a major and long lasting “fatal” anoxic insult.

In a separate protocol, the authors injected directly into the anteroventral preoptic region 13 femtomoles of Na₂S in a volume of 0.1 microl corresponding therefore to a much lower

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Conflict of Interest There is no conflict of interest to declare.

concentration (0.130 microM). They also found a reduction of the response to hypoxia following this injection.

Small mammals have an exquisite sensibility to moderate hypoxia (Mortola, 1993, Frappell et al., 1991, Hinrichsen et al., 1998, Haouzi et al., 2009) and to low levels of sulfide (Haouzi et al., 2009) as far as their ability to reduce the metabolic activity/temperature is concerned. Does this imply that some of the neurons involved in the regulation of non-shivering thermogenesis could have, just like for the chemoreceptor cells (Buckler and Turner, 2013, Buckler, 2012), the ability to modify their mitochondrial transmembrane potential, and reduce ATP accordingly, in response to moderate levels of hypoxia and H₂S? This would fit with the observation that the inhibitory effect on the response to hypoxia was still observed 30 minutes after the end of H₂S injection, at a time when the persistence of free/gaseous H₂S is very unlikely. The fate of H₂S in tissues in vivo is still to be clarified (Lagoutte et al., 2010), but neurons appear to be much more vulnerable to exogenous H₂S than any other cells. The determination of the minimal level of H₂S at which the mitochondrial ATP production is impeded in various neurons remains therefore an outstanding question (Bouillaud and Blachier, 2011). For instance, H₂S-induced CNS toxicity leads to reversible episodes of unconsciousness typically refers to as "knockdown" (Guidotti, 1994) without affecting breathing control. This suggests that concentrations of soluble H₂S within the high nanomolar range may already reduce the mitochondrial function of neurons located in supra-bulbar regions.

An increasing number of studies have been using microinjection of H₂S in various animal models at different concentrations in an attempt to mimic the putative effects of endogenous H₂S. This approach remains an important step to investigate the physiological role of H₂S since 1) agents inhibiting CBS activity, such as AOA, are not only altering H₂S production but can also affect methylation processes, as well as methionine and homocysteine metabolism and 2) methods measuring H₂S in tissues, using for instance the formation of methylene blue, can be extremely misleading (Olson, 2011, Van de Louw and Haouzi, 2012). Yet, unless proven otherwise, the use of microinjections of H₂S at concentrations mimicking the effects of a local anoxia/hypoxia or leading to post hypoxic injury could shift the topic of any physiological study to questions related to H₂S poisoning or neuronal dysfunctions in response to an anoxic insult. It would be essential to establish for any neuronal system of interest whether or the concentrations of H₂S used are affecting mitochondrial ATP production, otherwise it could well be the mechanisms of toxicity of H₂S that are investigated.

Acknowledgements

This work was supported by the CounterACT Program, National Institutes of Health Office of the Director (NIH OD), and the National Institute of Neurological Disorders and Stroke (NINDS), Grant Number 1R21NS080788-01.

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