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REVIEW ARTICLE

Hydrogen sulfide pathway and skeletal muscle: an introductory review

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The presence of the H_2S pathway in skeletal muscle (SKM) has recently been established. SKM expresses the three constitutive H_2S-generating enzymes in animals and humans, and it actively produces H_2S. The main, recognized molecular targets of H_2S, that is, potassium channels and PDEs, have been evaluated in SKM physiology in order to hypothesize a role for H_2S signalling. SKM dysfunctions, including muscular dystrophy and malignant hyperthermia, have also been evaluated as conditions in which the H_2S and transsulfuration pathways have been suggested to be involved. The intrinsic complexity of the molecular mechanisms involved in excitation-contraction (E-C) coupling together with the scarcity of preclinical models of SKM-related disorders have hampered any advances in the knowledge of SKM function. Here, we have addressed the role of the H_2S pathway in E-C coupling and the relative importance of cystathionine β-synthase, cistathionine γ-lyase and 3-mercaptopyruvate sulfurtransferase in SKM diseases.

Abbreviations

MPST, 3-mercaptopyruvate sulfurtransferase; CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; DMD, duchenne muscular dystrophy; E-C, excitation-contraction; Hcy, homocysteine; HHcy, hyperhomocysteinaemia; K_{ATP}, potassium ATP; K_v7, voltage-dependent potassium; MHS, malignant hyperthermia susceptible; RyR1, ryanodine 1 receptor; SKM, skeletal muscle

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Introduction

The function of skeletal muscle (SKM) is governed by a process known as excitation-contraction (E-C) coupling. This physiological phenomenon has intrigued many scientists since the XVIII century; however, only in the past 50 years have the molecular mechanisms involved, some structural components and the sequence of events required for muscle contraction been identified.

In the striated muscle, Ca^{2+} release and uptake can be considered as the 'key point' of E-C coupling (Eshima *et al.,* 2014). We do not yet have a clear understanding of the molecular interactions that allow the E-C coupling signal; however, a macromolecular protein complex defined as a 'calcium release unit' has been identified (Dulhunty, 2006; Bellinger *et al.,* 2008). Skeletal isoforms of the **dihydropyridine receptor, L-type Ca^{2+} channel** ($Ca_v1.1$) and ryanodine 1 receptor (**RyR1**) constitute the main structures of the 'calcium release unit', and their physical interaction is an essential requirement for E-C coupling in SKM (Dulhunty *et al.,* 2002). However, several other proteins are involved in the skeletal E-C coupling such as calmodulin, calstabin, A-kinase anchor protein, cAMP-dependent PKA, Ca^{2+}/calmodulin-dependent protein kinase II and **PDE4D3** (Bellinger *et al.,* 2008; Arias-Calderon *et al.,* 2016). Dystrophin and syntrophin are also involved in the molecular machinery of E-C coupling. Indeed, their malfunctioning, due to an erroneous assembly, has been hypothesized as a cause of muscle fibre necrosis – one of the histological features typical of muscular dystrophies (Finkel *et al.,* 2010; Flanigan, 2014). Among the proteins involved in E-C coupling, RyR1 is probably the most studied; three mammalian genes, encoding the following different isoforms, have been identified: SKM RyR1, cardiac muscle ryanodine receptor (RyR2) and RyR3 that is not tissue-specific, but instead is widely expressed in the body (Lai *et al.,* 1988 Meissner *et al.,* 1988; for details on RyR molecular structure, see Zalk *et al.,* 2015). Membrane depolarization triggers RyR1 opening with the consequent exit of Ca^{2+} ions from the sarcoplasmatic reticulum to the cytosol increasing the intracellular Ca^{2+} concentration from 10^{-7} to 10^{-5} M. This event leads to conformational changes that culminate in muscle contraction. In addition to cytosolic Ca^{2+} concentration, many regulatory factors have been identified and/or suggested as modulators of RyR activity, among which is the redox status of the receptor. Indeed, within each subunit of the RyR molecular structure, there are 80–100 **L-cysteine** residues, and almost 20 of them are sensitive to chemical modification such as sulphydryl oxidation, S-nitrosylation or alkylation that can promote either activation or inhibition of RyR activity (Dulhunty *et al.,* 2000; Pessah and Feng, 2000). However, although all these studies strongly suggest that hyperreactive sulfhydryl moieties are an essential biochemical component of a transmembrane redox sensor *in vitro*, how much this phenomenon could influence Ca^{2+} regulation *in vivo* and if its contribution varies between physiological and pathological conditions remains, as yet, unknown.

Hydrogen sulfide (H_2S) signal transduction and potential molecular targets in SKM

H_2S is the latest endogenous gasotransmitter to be discovered, and it is produced in several tissues and organ systems in animals and humans. It can be derived by both enzymatic and non-enzymatic pathways, although the non-enzymatic source accounts for only a minor portion of the H_2S generated in the body. In mammals, H_2S biosynthesis occurs in the cytosol and/or the mitochondria (see Wallace and Wang, 2015), and it is provided by different enzymes widely expressed throughout the body (Table 1). **Cystathionine β-synthase (CBS)** was the first H_2S-generating enzyme to be discovered and is the best characterized. CBS protein is considered the main source of H_2S in the CNS (Abe and Kimura, 1996; Eto and Kimura, 2002; Miles and Kraus, 2004); however, it is also abundantly expressed in peripheral tissues (Hosoki *et al.,* 1997; Fiorucci *et al.,* 2006; Szabo *et al.,* 2013; Zhang *et al.,* 2013; Bucci *et al.,* 2014; Vellecco *et al.,* 2016). **Cystathionine γ-lyase (CSE** or CGL or CTH) is considered the main H_2S-generating enzyme in the vasculature. As with CBS, CSE expression has been found in many locations in the body (Zhao *et al.,* 2001; Fiorucci *et al.,* 2006; Bucci *et al.,* 2012; Vellecco *et al.,* 2016; Cirino *et al.,* 2017). CBS and CSE are both pyridoxal-5-phosphate dependent enzymes, and they use the amino acid L-cysteine as a substrate (Kabil and Banerjee, 2014; Kimura, 2014). Both proteins are involved in different reactions of the trans sulfuration pathway, some of which do not produce H_2S (Kabil and Banerjee, 2014). **3-Mercaptopyruvate sulfurtransferase (MPST)** is the third H_2S-generating enzyme; it is pyridoxal-5-phosphate-independent and needs 3-mercaptopyruvate as a substrate. MPST has been found in vasculature and in smooth muscle component of several organs (Nagahara *et al.,* 1998; Shibuya *et al.,* 2009; Modis *et al.,* 2013; Vellecco *et al.,* 2016). The discovery of H_2S as endogenous signalling molecule, and the localization of the enzymatic machinery responsible for its biosynthesis throughout the body, has prompted scientists to investigate the existence of an H_2S pathway in SKM and to evaluate its role in both physiological and pathological conditions. Not much literature is available on this specific topic. Nevertheless, some studies have shown that SKM expresses the three constitutive enzymes responsible for H_2S biosynthesis in rats (Chen *et al.,* 2010; Du *et al.,* 2013) and in humans (Islam *et al.,* 2015; Vellecco *et al.,* 2016). In terms of relative expression, the amount of these three proteins in rat SKM is significantly less compared to the amounts in the liver and kidneys (Du *et al.,* 2013). Conversely, the amounts of H_2S-generating enzymes in human SKM is comparable with those observed in the liver and kidneys (Islam *et al.,* 2015). In particular, by using fluorescent immunohistochemistry methods, Du *et al*. have found a different sub-localization of the enzymes within the rat muscle fibre. Indeed, CBS and MPST are mainly expressed in the endomysium and perimysium of SKM, whilst CSE is localized in the cytosol of the muscle fibre cells (Du *et al.,* 2013). However, the relative roles of each enzyme in muscle physiology and/or pathology has yet to be demonstrated. Unexpectedly, it has been shown that mouse SKM lacks all three enzymes (Chen *et al.,* 2010; Veeranki and Tyagi, 2015). Indeed, Veeranki *et al*. have shown that mouse SKM expresses methylenetetrahydrofolate reductase, an enzyme that participates in the re-methylation of **homocysteine** into methionine (see Figure 1), but it does not express CSE, CBS or MPST and, consequently, has a reduced L-cysteine and H_2S content. The authors hypothesized that the lack of these enzymes

Table 1

Localization of H_2S-generating enzymes

Enzymes	Tissues	References
CBS	Brain[h, m, r]	Martin *et al.*, 2009; Linden *et al.*, 2008; Bronowicka-Adamska *et al.*, 2017
	Gastrointestinal tract [h, m, r]	Martin *et al.*, 2009; Linden *et al.*, 2008
	Heart[m, r]	Testai *et al.*, 2016; Nandi and Mishra, 2017
	Kidney[m, r]	Ahmad *et al.*, 2016; Du *et al.*, 2013
	Liver[h, m, r]	Ahmad *et al.*, 2016; Du *et al.*, 2013; Martin *et al.*, 2009
	Lung[h, m, r]	Ahmad *et al.*, 2016; Martin *et al.*, 2009; Szczesny *et al.*, 2016
	Pancreas[m, r]	Ahmad *et al.*, 2016; Yusuf *et al.*, 2005
	Placenta[h, m, r]	Hu *et al.*, 2017; Patel *et al.*, 2009; Sonne *et al.*, 2013
	Skeletalmuscle [h, r]	Vellecco *et al.*, 2016; Du *et al.*, 2013
	Spleen[m]	d'Emmanuele di Villa Bianca *et al.*, 2009; Mitidieri *et al.*, 2016; Zhang *et al.*, 2016
	Uro-genital tract[h, m, r]	Brancaleone *et al.*, 2014
	Vasculature[m, r]	Bucci *et al.*, 2014; Bucci *et al.*, 2012
CSE	Brain[h, m, r]	Martin *et al.*, 2009; Linden *et al.*, 2008; Bronowicka-Adamska *et al.*, 2017
	Gastrointestinal tract[h, r, m]	Martin *et al.*, 2009; Linden *et al.*, 2008
	Heart[m, r]	Yang *et al.*, 2008; Testai *et al.*, 2016
	Kidney[m, r]	Yang *et al.*, 2008; Du *et al.*, 2013
	Liver[h, m, r]	Yang *et al.*, 2008; Du *et al.*, 2013; Martin *et al.*, 2009
	Lung[h,m, r]	Ahmad *et al.*, 2016; Chen *et al.*, 2009; Szczesny *et al.*, 2016
	Pancreas[m, r]	Yang *et al.*, 2008; Yusuf *et al.*, 2005
	Placenta[h, r]	Hu *et al.*, 2017; Patel *et al.*, 2009
	Skeletal muscle[h, r]	Vellecco *et al.*, 2016; Du *et al.*, 2013
	Spleen[m]	Brancaleone *et al.*, 2014
	Uro-genital tract[h, m, r]	d'Emmanuele di Villa Bianca *et al.*, 2009; Mitidieri *et al.*, 2016; Zhao *et al.*, 2016
	Vasculature[h, m, r]	Bucci *et al.*, 2014; Bucci *et al.*, 2012; Renga *et al.*, 2015
MPST	Brain[h, m, r]	Tomita *et al.*, 2016; Zhao *et al.*, 2013; Bronowicka-Adamska *et al.*, 2017
	Gastrointestinal tract[m, r]	Tomita *et al.*, 2016; Magierowski et al., 2017
	Heart[m, r]	Tomita *et al.*, 2016; Testai *et al.*, 2016
	Kidney[m, r]	Tomita *et al.*, 2016; Du *et al.*, 2013
	Liver[h, m, r]	Tomita *et al.*, 2016; Du *et al.*, 2013; Li *et al.*, 2017
	Lung[h, m]	Tomita *et al.*, 2016; Szczesny *et al.*, 2016
	Pancreas[m]	Tomita *et al.*, 2016
	Placenta[h]	Hu *et al.*, 2017
	Skeletal muscle[h, r]	Vellecco *et al.*, 2016; Du *et al.*, 2013
	Spleen[m]	Tomita *et al.*, 2016
	Thymus[m]	Tomita *et al.*, 2016
	Thyroid[m]	Tomita *et al.*, 2016
	Vasculature[h, m, r]	Shibuya *et al.*, 2009; Bucci *et al.*, 2014; Kuo *et al.*, 2016
	Uro-genital tract[m]	Aydinoglu *et al.*, 2017

Tissue localization of H_2S-generating enzymes (h = human; m = mouse; r = rat). The three enzymes involved in H_2S biosynthesis are widely distributed in tissues of human (h), rat (r) and mouse (m). CBS, cystathionine β-synthase; CSE, cystathionine γ-lyase; MPST, 3-mercaptopyruvate sulfurtransferase.

makes mouse SKM prone to hyperhomocysteinaemia (HHcy)-induced myopathy (Veeranki and Tyagi, 2015). However, further studies are needed to clarify this issue.

Activation of potassium channels

Being a gas, H_2S travels freely across cell membranes activating various molecular targets in a receptor-independent manner. This characteristic allows this gasotransmitter to be considered as a mediator in many processes in both physiological and pathological conditions (Fiorucci *et al.*, 2006; Lowicka and Beltowski, 2007; Szabo, 2007; Li *et al.*, 2011; Cirino *et al.*, 2017; Wallace and Wang, 2015). Among the known cellular targets of H_2S, potassium channels have been the first to be discovered. Activation of ATP-sensitive

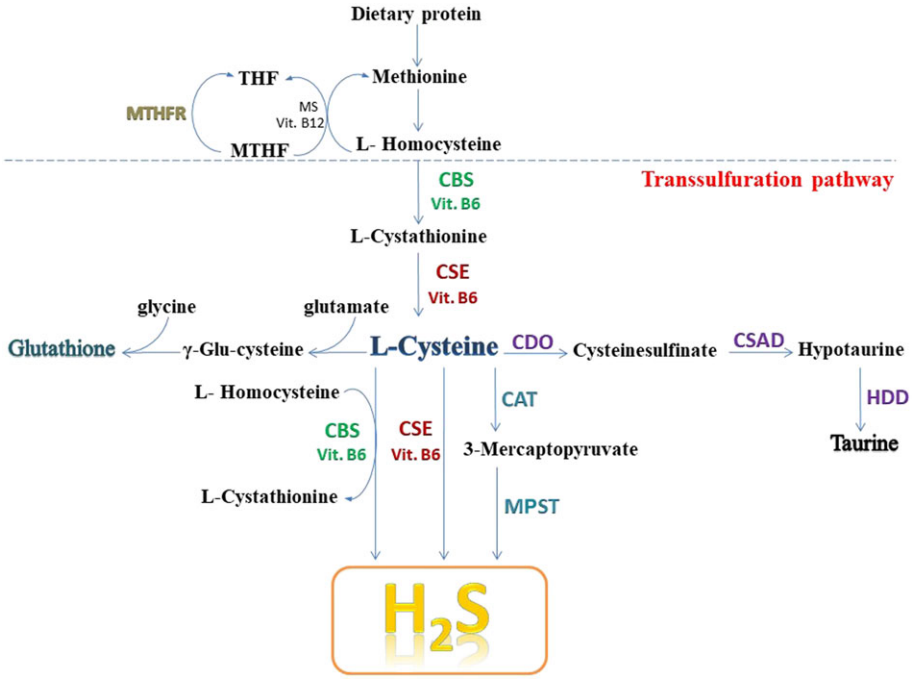

Figure 1

Simplified scheme of transsulfuration pathway. The mammalians introduce the aminoacid methionine with the diet. The methionine could be converted into homocysteine and back to methionine. Homocysteine acts as a substrate leading to the synthesis of three major final products: H_2S, taurine and glutathione. **CAT**, cysteine aminotransferase; CDO cysteine dioxygenase; CSAD, cysteine sulfonic acid decarboxylase; CSE, cystathionine γ-lyase; HDD, hypotaurine dyhydrogenase; MPST, 3-mercaptopyruvate sulfur transferase; MTHFR, methylenetetrahydrofolate reductase.

potassium (**K_{ATP}**) channels (Zhao *et al.*, 2001; Tang *et al.*, 2005; Distrutti *et al.*, 2006; Dawe *et al.*, 2008; Jiang *et al.*, 2010; Medeiros *et al.*, 2012; Fitzgerald *et al.*, 2014), small- and intermediate-conductance **calcium-activated potassium channels** (SKCa channels and IKCa channels, respectively) (Tang *et al.*, 2013, Mustafa *et al.*, 2011; Telezhkin *et al.*, 2010; Li *et al.*, 2010) and, more recently, **voltage-dependent potassium (K_V7) channels** (Martelli *et al.*, 2013; Hedegaard *et al.*, 2014) account for different biological functions of H_2S in the cardiovascular, nervous, respiratory, gastrointestinal and endocrine systems. The activation of H_2S-stimulated K_{ATP} channels has been demonstrated by using a whole-cell patch-clamp technique and a mutagenesis approach: in colonic smooth muscle cells. H_2S specifically S-sulfhydrates the sulfonylurea receptor 1 (SUR1) and SUR2B subunits of K_{ATP} channels leading to an increased K_{ATP} channel current (Jiang *et al.*, 2010). A similar mechanism of S-sulphydration of K_{ATP} channels has also been shown in vascular smooth muscle cells where the increased amplitude of the K_{ATP} channel current leads to hyperpolarization (Tang *et al.*, 2005; Mustafa *et al.*, 2009). In mouse SKM sarcolemma, K_{ATP} channels are very highly expressed (Flagg *et al.*, 2010; MacIntosh *et al.*, 2012), and, despite the fact that their physiological function in SKM is not so clearly defined as in cardiac tissue, K_{ATP} channel activation has been suggested to be involved in the prevention of calcium overload and preservation of myofibre integrity during exercise, as well as recovery from muscle fatigue, rather than in normal muscle contractility and excitability (Matar *et al.*, 2000; MacIntosh *et al.*, 2012). Since H_2S is actively produced in human SKM, and is detectable in nanomolar range (Vellecco *et al.*, 2016), it is feasible that H_2S could modulate the activation of K_{ATP} channels during muscle activity. As demonstrated in smooth muscles, it is possible that H_2S induces K_{ATP} channel activation through a mechanism involving S-sulfhydration. In this context, it is interesting to note that in patients susceptible to malignant hyperthermia (MHS), a syndrome characterized by a diffused hyper-contractility of SKM induced by volatile anaesthetics, the content of H_2S in the *vastus* muscle is ~10-fold higher compared to healthy subjects. The hyper-contractility in halothane-induced MHS is significantly reduced by glibenclamide, a selective blocker of K_{ATP} channels (Vellecco *et al.*, 2016). This finding suggests that the increased amount of H_2S detected in MHS subjects contributes to the anomalous contraction elicited by volatile anaesthetics. This hypothesis is corroborated by the finding that the mechanism of action of volatile anaesthetics involves the activation of several channels, including K_{ATP} channels (Yoo *et al.*, 2006; Matchett *et al.*, 2009). So it is feasible to speculate that in SKM of MHS subjects, there is a constitutive hyper-activation of K_{ATP} channels due to increased levels of H_2S. When these subjects are challenged with volatile anaesthetics, a state of 'over-activation' of K_{ATP} channels takes place, contributing to the characteristic SKM hyper-contractility of MHS subjects.

Other H_2S molecular targets with a recognized role in the function of SKM are the sub-class of voltage-dependent potassium K_v7 channels. It is known that K_v7 channels are

involved in resting membrane potential and electrical excitability control in many cell types included SKM (Miceli *et al.,* 2008; Roura-Ferrer *et al.,* 2008). In particular, it has been shown that in mouse C_2C_{12} cells, **K_v7.4** channels have a crucial role in the induction and/or maintenance of the differentiated state in skeletal myotubes (Iannotti *et al.,* 2013). The same group also demonstrated that K_v7 channels regulate the response of SKM to myotoxic stimuli, suggesting that K_v7 channel modulators have potential as new therapeutic agents for myopathies (Iannotti *et al.,* 2010). Recently, Martelli *et al*. have added K_v7 channels as new molecular targets for H_2S (Martelli *et al.,* 2013). In particular, they showed that, in isolated mouse aorta, the vasorelaxing effect of NaHS (used as an exogenous source of H_2S) is significantly inhibited by **XE-991**, a selective K_v7 channel blocker. In addition, exposure of human aortic smooth muscle cells to NaHS promotes membrane hyperpolarization to a similar extent to that observed for the selective K_v7 channel opener **retigabine**, and this effect was significantly antagonized by both selective blockers of K_v7 and K_{ATP} channels (Martelli *et al.,* 2013). All these data could suggest a role for a H_2S/K_v7 interaction in SKM function.

Phosphodiesterase inhibition

It has been demonstrated that in the vascular system, H_2S acts as an endogenous non-selective inhibitor of the activity of **PDEs** (Bucci *et al.,* 2010; Bucci *et al.,* 2012; Coletta *et al.,* 2012). PDEs are a class of metalophosphohydrolases that, by hydrolyzing the cyclic nucleotides (**cGMP** and **cAMP**), regulate their physiological levels. H_2S, by inhibiting the activity of PDEs, slows down the degradation rate of cyclic nucleotides modulating the transduction of downstream signals that involve cAMP and cGMP as second messengers. Within the vasculature, inhibition of **PDE5**, with the consequent increase in cGMP, is one of the main mechanisms of H_2S-induced vasorelaxation (Bucci *et al.,* 2010). In SKM, different PDE isoforms have been found: PDE4(B,C,D), PDE7A and PDE8B, which selectively hydrolyze cAMP; PDE5A, which selectively hydrolyzes cGMP; and PDE11, which hydrolyzes both cyclic nucleotydes (Nio *et al.,* 2017; Tetsi *et al.,* 2017). However, much more information is available on cAMP metabolism compared to cGMP in SKM (Stapleton *et al.,* 2014; Nio *et al.,* 2017). Nevertheless, it has been shown that in *mdx* mice, the most widely used murine model of duchenne muscular dystrophy (DMD), treatment with the selective PDE5 inhibitor **sildenafil** reverses the cardiac dysfunction (Adamo *et al.,* 2010) and reduces respiratory muscle weakness and fibrosis (Percival *et al.,* 2012), which are typical features of DMD. More recently, Nio *et al*. have shown that the treatment of *mdx* mice with piclamilast, a PDE4-selective inhibitor, also displays an antifibrotic effect and the combination therapy of piclamilast and sildenafil further increases the antifibrotic effect observed (Nio *et al.,* 2017). The evidence that, in SKM, H_2S is actively produced and different isoforms of PDEs are constitutively expressed allows us to speculate that H_2S, by inhibiting the activity of PDEs, could also modulate the level of cyclic nucleotides in SKM (see Figure 2).

Potential role of CBS and/or CSE in SKM dysfunctions

It is well established that the lack/impairment of CBS activity is the most common cause of hyperhomocysteinaemia (HHcy; for review see Morris *et al.,* 2017). HHcy represents a risk factor for several human diseases, and patients with HHcy manifest some characteristic features (early thrombotic events and cognitive decline) including SKM dysfunctions. As CBS is a H_2S-generating enzyme, a deficiency in CBS provides a clear link between hydrogen sulfide and the pathophysiology of SKM. Indeed, several studies have correlated HHcy with SKM dysfunction. In 1976, Kanwar *et al*. showed that the SKM of patients with homocystinuria is characterized by anomalous collagen deposits in the basal lamina, which was associated with a disrupted Z-line (Kanwar *et al.,* 1976). This finding has been recently confirmed in a preclinical setting where chronic administration of homocysteine to rats caused a reduction in the viability of SKM cells and produced an energy imbalance (Kolling *et al.,* 2013). Also, some neurological pathologies that induce muscle degeneration, such as amyotrophic lateral sclerosis and multiple sclerosis,

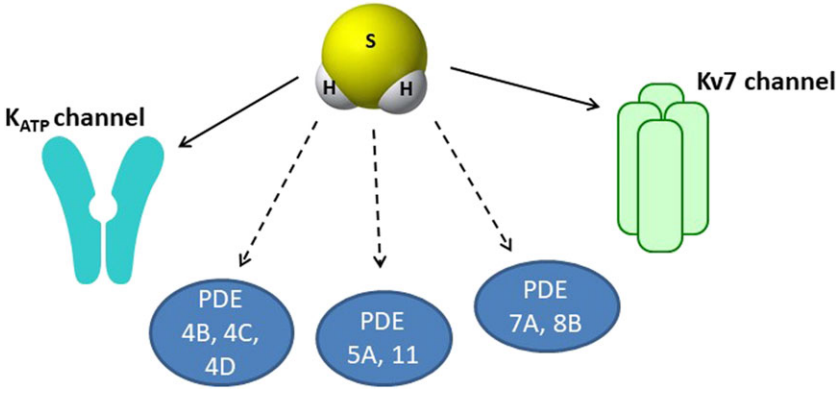

Figure 2

Potential targets of hydrogen sulfide (H_2S) in SKM. Different recognized, molecular targets of H_2S that are expressed physiologically in SKM: ATP-sensitive potassium (K_{ATP}) and voltage dependent (K_v7) channels. Several PDEs that are potential targets for H_2S are also reported. Solid line: activation; dotted line: inhibition.

have been correlated with high levels of homocysteine in plasma and cerebrospinal fluids (Valentino *et al.,* 2010; Zoccolella *et al.,* 2012). The molecular mechanisms of the deleterious action of homocysteine on SKM have not yet been clearly defined. It has been hypothesized that the availability of homocysteine in the muscle fibre is reduced due to the fact that it competes with the L-cysteine transporters (Veeranki and Tyagi, 2015). Such a reduction in L-cysteine content not only affects the amount of H_2S generated locally but also promotes oxidative stress, since L-cysteine is the precursor not only of H_2S but also of glutathione and taurine, two of the main endogenous antioxidants (Stipanuk, 2004; Veeranki and Tyagi, 2015). This mechanism has been shown to be present in a preclinical rat model of HHcy where an increase in ROS production was found to be associated with reduced glutathione levels (Kolling *et al.,* 2013). Similarly, a significant decrease in H_2S content in muscle fibre, together with a decrease in SOD1 expression, is present in a rat model of SKM ischaemia–reperfusion (I-R) injury (Du *et al.,* 2013). These findings are associated with an increase in ROS (H_2O_2 and O_2^-) and malondialdehyde (MDA) production leading to severe necrosis, as revealed by histopathological analysis of the gastrocnemius muscle. It is noteworthy that pretreatment with NaHS decreases MDA content, reduces hydrogen peroxide and superoxide anion levels, but increases SOD activity and protein expression thereby protecting the SKM from oxidative stress (Du *et al.,* 2013). This latter finding strongly suggests that: (i) being an antioxidant molecule, H_2S exerts a protective effect on SKM maintaining the physiological levels of oxidative products; (ii) the impairment of the endogenous production of H_2S in SKM induces a disequilibrium in redox status with a consequent increase in ROS production that damages muscle fibres. The oxidative stress is further worsened by the reduction in SOD expression induced by I-R injury; and (iii) the exogenous administration of H_2S not only regains the redox status but also restores SOD expression with the final effect of protecting SKM from oxidizing agents. In line with this view, it has been hypothesized that that a dysregulation of H_2S metabolism is involved in chronic fatigue syndrome, also called myalgic encephalomyelitis (Dix Lemle, 2009). This is a very debilitating disease with an unknown aetiology and heterogeneous symptoms in terms of intensity, appearance and duration that makes diagnosis difficult (Collatz *et al.,* 2016). Post-exertional malaise, muscle and joint pain, difficulties with short-term memory, unrefreshing sleep, sore throat and headaches are some of a pattern of symptoms of this syndrome, often associated with dysregulation of body temperature and blood pressure (Collatz *et al.,* 2016). It has been suggested that a systemic dysfunction of H_2S metabolism is the main cause of chronic fatigue syndrome. Such a dysregulation could explain the pattern of symptoms, apparently unconnected, typical of chronic fatigue syndrome (Dix Lemle, 2009).

A role of altered levels of H_2S in SKM has been also present in SKM bundles harvested from MHS patients. In particular, molecular analysis shows that the main source of H_2S in MHS patients is CBS that results strongly over-expressed, in terms of both protein and mRNA, compared to MHN. These evidence suggest that CBS-derived H_2S is the main source of the 'pathological' high levels of H_2S in MHS subjects (Vellecco *et al.,* 2016).

Another clue as to the role of H_2S signalling in SKM function arises from the study by Ishii *et al.* (2010). In this study, $CSE^{-/-}$ mice were generated as an animal model of cystathioninemia/cystathioninuria, a known autosomal recessive inborn error with increased plasma/urinary levels of cystathionine, with no pathological phenotype (Mudd *et al.,* 2001). The study shows that by feeding $CSE^{-/-}$ mice a low cysteine diet from 3 weeks old, an acute myopathy occurs, which is associated with a reduced concentration of glutathione in SKM and the liver. In more detail, once the low L-cysteine diet starts, the mice promptly lose weight whilst WT mice and $CSE^{-/-}$ mice fed a standard diet keep growing. Then, the mice display a paralysis of their lower extremities and severe atrophy in the abdominal regions, the trapezius and rectus femoris muscles. Thereafter, the mice become lame, paralysed in the upper extremities and finally die. Histological analysis of femur SKM sections of $CSE^{-/-}$ mice fed the low L-cysteine content revealed the intracellular accumulation of the autophagosomal marker LC3 and p62/sequestosome-1 in skeletal myofibres, suggesting enhanced autophagy leading to myopathic muscle loss (Masiero *et al.,* 2009). These findings strongly suggest a pivotal role for CSE in the development and function of SKM even though the exact contribution of this enzyme to the onset of myopathy is unknown.

Conclusions

Considering the enormous advances in life sciences made in recent decades, it is surprising, at least at first sight, how much there is still to clarify as regards SKM function. There are several hurdles to overcome: (i) SKM dysfunction ranges from generic muscle weakness and soreness to severe myopathy, muscle wasting and cachexia. Often, these signs are not directly correlated with a specific SKM disease, that is, muscular dystrophy, but rather constitute one symptom of a more complex syndrome that does not necessarily originate from SKM; (ii) despite the fact that many studies have been devoted to clarifying the molecular mechanisms involved in E-C coupling *in vitro*, the real impact of these mechanisms are very difficult to assess *in vivo* because of the intrinsic complexity of this phenomenon; and (iii) the information gleaned from animal models of muscle diseases does not always translate to human pathology. In addition, for many SKM human pathologies, a suitable animal model is still lacking.

An interesting speculation that suggests a relevant role for the involvement of the L-cysteine/CSE-CBS/H_2S pathway in the physiopathology of SKM relies on two findings that have been discussed within this review: (i) the lack of CSE genes, coupled with a low cysteine diet, induces an acute myopathy characterized by paralysis of the lower extremities and severe atrophy (Ishii *et al.,* 2010); (ii) in human subjects susceptible to malignant hyperthermia, there is a hyper-contractility of SKM accompanied by an increased local production of H_2S coupled with an overexpression of CBS (Vellecco *et al.,* 2016). Therefore, whilst a lack of CSE and the low availability of L-cysteine causes an impairment of the function of SKM, an increase in CBS expression and consequent enhanced production of hydrogen sulfide leads to muscle hyper-reactivity.

From this point of view, it appears clear that the murine model of DMD, that is, mdx mice, displays some similarities to CSE $^{-/-}$ mice fed a low cysteine content diet. Indeed, slow and progressive muscle weakness coupled with muscle degeneration are some of the features of mdx mice (Willmann *et al.,* 2009; Manning and O'Malley, 2015; McGreevy *et al.,* 2015). This hypothesis is further supported by the study of Terrill *et al*. where it has been reported that the treatment of adult mdx mice with an L-cysteine precursor reduces the dystropathology and oxidative stress in these mice (Terrill *et al.,* 2013). Therefore, it is hypothesized that low levels of H_2S (plasma or tissue levels) are an index of reduced SKM performance, whilst high levels are an index of hypercontractility/susceptibility. In conclusion, there is much to do in order to understand the role of hydrogen sulfide in the physiopathology of SKM. However, the nature of this gasotransmitter makes it an ideal player in the physiology of SKM, as among its targets there are channels and enzymes that are known to play an important role in the homeostasis of SKM.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.,* 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017a,b).

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Conflict of interest

The authors declare no conflicts of interest.

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