Implications of Hydrogen Sulfide in Glucose Regulation: How H₂S Can Alter Glucose Homeostasis through Metabolic Hormones

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Diabetes and its comorbidities continue to be a major health problem worldwide. Understanding the precise mechanisms that control glucose homeostasis and their dysregulation during diabetes are a major research focus. Hydrogen sulfide (H₂S) has emerged as an important regulator of glucose homeostasis. This is achieved through its production and action in several metabolic and hormone producing organs including the pancreas, liver, and adipose. Of importance, H₂S production and signaling in these tissues are altered during both type 1 and type 2 diabetes mellitus. This review first examines how H₂S is produced both endogenously and by gastrointestinal microbes, with a particular focus on the altered production that occurs during obesity and diabetes. Next, the action of H₂S on the metabolic organs with key roles in glucose homeostasis, with a particular focus on insulin, is described. Recent work has also suggested that the effects of H₂S on glucose homeostasis go beyond its role in insulin secretion. Several studies have demonstrated important roles for H₂S in hepatic glucose output and adipose glucose uptake. The mechanism of H₂S action on these metabolic organs is described. In the final part of this review, future directions examining the roles of H₂S in other metabolic and glucoregulatory hormone secreting tissues are proposed.

1. Introduction

Hydrogen sulfide (H₂S) is a colorless and odorless gas that is produced both endogenously by a variety of mammalian cells and by the sulfate reducing bacteria in the lower gastrointestinal (GI) tract. H₂S has emerged as an important gasotransmitter that regulates several systems including the cardiovascular, GI, immune, endocrine, and nervous systems (reviewed in detail in [1]). One area of recent interest is the potential role that H₂S may play in glucose regulation and metabolic health. Indeed, several groups have demonstrated that obese and diabetic individuals have altered H₂S levels in their circulation [2, 3] and tissues [4, 5]. The precise mechanisms of how H₂S can drive metabolic changes are beginning to be understood. A major factor in the regulation of glucose metabolism is the secretion and action of metabolic hormones. These hormones include insulin, glucagon, leptin, and glucagon like peptide-1. Several groups have already described the action of H₂S on insulin secretion [6–8]. Furthermore, recent work has demonstrated the effects of H₂S on downstream hormone signaling [9]. These studies and others suggest that H₂S may be a potential target in the treatment of metabolic diseases through modulating metabolic hormone secretion and signaling. The goal of this review is to describe the roles of H₂S in the regulation of metabolic hormone secretion, with a particular focus on insulin, and the downstream signaling of these hormones in the regulation of energy homeostasis.

2. H₂S Production

Although the presence of H₂S in the body has been known for some time, the precise locations of its production remain an active area of research. H₂S is produced by a large variety of cell types in the body (here named endogenous) and by host microbes including the sulfate reducing
bacteria in the GI tract. The main enzymatic machineries in the endogenous production of H$_2$S are the cystathionine metabo-
lizing cystathionine-$\beta$-synthase (CBS) [10] and cystathionine $\gamma$-lyase (CSE) [11]. Other enzymes such as 3-mercapto-
pyruvate sulfurtransferase (MST) and cysteine aminotransferase (CAT) are also important in specific tissue
types [12]. CSE activity is much higher than CBS in peripheral
tissues, while CBS mainly predominates in the brain [13, 14]. The precise mechanisms involving the production of endo-
enous H$_2$S are thoroughly reviewed by Wang in [1]. Once H$_2$S is produced in the cell, it can act on different
cellular pathways or be stored for later release. H$_2$S can store its sulfur group with iron (acid labile sulfur) [15] or in
sulfane sulfur (a persulfide) [16] in mammalian tissues. When
required and under the appropriate conditions, this bound
sulfur can be released as $S^{2-}$, $HS^-$, or H$_2$S [17].

In addition to endogenous generation, H$_2$S can be pro-
duced from microorganisms in the GI tract. The gut micro-
biotia aids in the decomposition and harvest of nutrients
from food, a crucial step in energy production. Primary
fermenters break down protein and complex carbohydrates
into short-chain fatty acids (e.g., acetate, propionate, and
butyrate) that are an important energy source, and gases
(e.g., hydrogen, carbon dioxide) that are released or absorbed
by the system. Hydrogenotrophs, or H$_2$-consuming bacteria,
are essential in keeping luminal hydrogen levels low and
stabilizing the environment for these primary fermenters.
Among the groups of hydrogenotrophs are methanogens
(producing methane), acetogens (producing acetate), and
sulfate reducing bacteria (producing H$_2$S). Sulfate reducing
bacteria use hydrogen or organic compounds as electron
donors and use sulfate as their terminal electron acceptor
leading to a large production of H$_2$S. This process is known as
dissimilatory sulfate reduction and can lead to mM concen-
trations of H$_2$S in the lumen [18]. Sulfur sources from diet
can originate from amino acids, preservatives, and food additives
carrageenan) or as dietary supplements (chondroitin sulfate)
[18]. Microbial produced H$_2$S is a significant contributor to
the bodies H$_2$S pool, as germ free mice have between 50 and
80% less H$_2$S in their tissues and circulation [19]. Microbial
H$_2$S has been associated with both maintaining gastric health
and being implicated in disease. Several groups have shown
that H$_2$S regulates various physiological functions including
maintenance of GI barrier function and injury repair [20].
Some earlier studies have suggested that H$_2$S may be involved
in the etiology of ulcerative colitis [21]. However, more
recent work points towards a protective role [22]. Regardless
of its source, H$_2$S has emerged as a regulator of glucose
metabolism. The mechanisms of this action are described
below.

3. Importance of H$_2$S in Diabetes and
Insulin Regulation

Insulin is one of the most researched and clinically important
metabolic hormones. Strategies that seek to enhance insulin
secretion and sensitivity are the cornerstone of diabetes treat-
ment. Insulin biosynthesis is regulated by many physiological
events; however the main driver of its secretion is circulating
glucose, such that, after a meal is consumed, the levels of
insulin spike in circulation. Insulin then acts on a variety of
tissues in the body, including, but not limited to, adipose,
liver, and muscle. The target cells are activated through the
insulin receptor which then leads to increased translocation
of glucose transporters to the membrane and glucose uptake.
During the development of type 2 diabetes mellitus (T2DM),
insulin signaling in the target tissues is impaired, and in order
to overcome this resistance, the $\beta$ cells of the pancreas begin
to proliferate and produce more insulin. In cases where the
pancreas is unable to produce sufficient insulin to regulate
the rising glucose levels, T2DM develops. In this scenario,
a variety of treatments that act to increase insulin levels
or enhance insulin signaling are employed. Nevertheless,
additional strategies to enhance insulin levels and signaling
are of great interest in the treatment of diabetes and metabolic
disease.

The investigation of hydrogen sulfide's potential involve-
ment in glucose metabolism began in 1990 when Hayden
and colleagues showed that H$_2$S exposure (2.2 mM) increased
circulating glucose in postpartum rats [23]. Later on, sev-
eral groups began to investigate how H$_2$S levels fluctuate
in metabolic disease. Human studies that have examined
circulating H$_2$S in T2DM have found them to be reduced.
Jain and colleagues found that T2DM individuals had sig-
ificantly lower H$_2$S compared to age matched nondiabetics
[2]. Whitman and colleagues confirmed these findings and
further demonstrated that adiposity was negatively correlated
with H$_2$S [3]. This is of particular interest since obesity is
one of the principal causes of T2DM. Unfortunately, the
mechanisms driving these changes in circulating H$_2$S, or
their effects on glucose metabolism, were not investigated.
As such, it is unclear whether the altered circulating H$_2$S
observed in obese individuals is a driving force in their
metabolic disease. A more mechanistic understanding of how
H$_2$S can alter glucose metabolism has come to light through
the examination of glucoregulatory hormones such as insulin
and its target tissues. These pathways and their role in glucose
homeostasis are described below.

4. H$_2$S Production and Function in
the Pancreas

The first evidence that H$_2$S was produced in the pancreas
and that it played a role in the regulation of insulin secretion
came from Yang and colleagues. Using the INS-1 cell line, they
demonstrated that $\beta$ cells express the enzymatic machinery
required to produce H$_2$S, including CSE, and can produce
high levels of H$_2$S which blocks glucose-stimulated insulin
secretion [8]. This was later confirmed in another $\beta$ cell
model, Min6 [24]. Yang and colleagues also demonstrated
that treating INS-1 cells with H$_2$S, or overexpressing CSE,
stimulated apoptosis [7]. This latter effect appeared to be
caused by increased endoplasmic reticulum stress and may be
a driving factor in the reduced insulin secretion observed
[7]. In addition, other groups have demonstrated the mRNA
expression of both CSE and CBS in the rat pancreas and that
streptozocin-induced diabetes (a model of type 1 diabetes)
causes increased mRNA expression of CBS and increased H$_2$S
production [4]. Using a rodent model of obese diabetes (the Zucker diabetic fatty rat), Wu and colleagues demonstrated that the animals impaired glucose metabolism was due to an overproduction of pancreatic H₂S and impaired insulin secretion [6]. Together, these studies suggest that increases in H₂S may be responsible for a reduction in insulin secretion and ultimately the impaired glucose clearance that occurs in diabetes. However, other groups have suggested that the elevated H₂S production from the β cell is occurring as a result of elevated circulating glucose and that H₂S is acting as a pancreatic brake, which may protect these insulin producing cells from being overstimulated by chronic hyperglycemia [25]. Indeed, it was later demonstrated that mice on a high fat diet lacking CSE have significantly worse islet glucotoxicity compared to WT animals [26]. This protective role for H₂S in β cell apoptosis occurs through H₂S mediated activation of thioredoxin, a system responsible for controlling redox homeostasis that protects β cells from glucotoxicity. The difference in reports of the protective versus toxic effect of H₂S in the pancreas may be due to the cell/animal model being used (whole animal versus cell studies and type I versus type 2 diabetes models). The differences in H₂S concentrations used would warrant further research into what concentration threshold is protective or detrimental to cellular function. Nevertheless, H₂S is produced in the pancreas and this appears to have important implications in insulin secretion and glucose homeostasis. How this gasotransmitter can elicit its effects on the cell is discussed below.

5. Mechanism of H₂S Action in the Pancreas

The earliest reports on the intracellular target of H₂S in insulin regulation were found to be an opening of the K_ATP channel [8]. When glucose enters the β cell, it generates ATP causing the closure of ATP sensitive K_ATP channels and opening of calcium channels leading to depolarization and thus insulin secretion [27]. When K_ATP channels are kept open by H₂S, the β cell is hyperpolarized and insulin secretion is suppressed. Based on this, several groups have demonstrated that compounds that suppress the production of H₂S can increase the secretion of insulin from β cells [8, 24]. The precise mechanisms that cause the opening of this channel remain an active area of research. It has been suggested that direct binding of H₂S to cysteine residues in proteins (sulfhydration) may be a potential mechanism [28]. Using the patch clamp method coupled with channel subunit mutagenesis, Jiang and colleagues demonstrated the importance of the rVKir6.1/rvSUR1 subunits in mediating K_ATP Channel opening [29]. It should be noted however that the above studies on the precise mechanisms of H₂S on the K_ATP have not been done in the β cell.

Voltage-dependent calcium channels (VDCCs) in the β cells control the movement of calcium, a crucial step in glucose-stimulated insulin release. One of the early studies examining the effect of H₂S in β cells found that NaHS (an H₂S donor) caused a decrease in the calcium oscillations caused by glucose, which ultimately led to reduced insulin secretion [24]. Using whole mouse islets, Tang and colleagues demonstrated (via patch clamp) that L-type VDCC current density is inhibited by the H₂S donor NaHS and that islets from mice lacking CSE had reduced L-type VDCC activity [29]. Of interest, these reports of decreased VDCC activity in β cells and islets are in contrast to the increased calcium concentrations that result from H₂S in cerebellar granule neurons [30]. This difference suggests that H₂S may regulate similar intracellular pathways in distinct manners depending on the cell type.

In addition to ion channel activities, H₂S may also regulate insulin secretion through the modulation of intracellular kinases. Several of these kinases are known to be modulated during the secretion of insulin including P38, ERK, AKT, and MAPK. Indeed, both endogenous and exogenous H₂S have been shown to directly activate the p38 MAPK [7]. Importantly, activation of the MAPK/JNK pathway is a known mechanism in impaired insulin release from the β cell [31]. More studies are required to determine if additional cell signaling pathways are altered through the activity of H₂S.

6. H₂S Effects on Metabolic Tissues

The description thus far focused on the production and effects of H₂S in the insulin secreting β cell. A vital part of glucose homeostasis is the function of the insulin sensitive metabolic organs, including adipose tissue, liver, and muscle.

One of the principle targets of insulin is the adipocyte. Insulin promotes the storage of excess glucose and its conversion to fat, leading to increased adiposity, a major risk factor for the development of metabolic disease. Several groups have demonstrated that adipose tissue produces H₂S, and that gasotransmitter production and signaling in the adipocyte are altered during obesity. Feng and colleagues were the first group to describe the expression of CBS and CSE and production of H₂S from rat adipocytes [32]. In this report they demonstrated that H₂S impairs insulin mediated glucose uptake and that high fructose-induced diabetes led to increased production of H₂S in epididymal adipose tissue, an effect that could be blocked by inhibiting CSE. This result points towards a negative effect of H₂S on glucose uptake in the adipocyte. Interestingly, circulating levels of H₂S are lower in obese humans [3], suggesting a disconnection in the increased production observed in the rodent adipose tissue. Some groups have demonstrated a positive role for H₂S in glucose metabolism in the adipocyte. One study in 3T3L1 adipocytes found that H₂S is required for vitamin D induced GLUT4 translocation and glucose uptake [33]. Another positive role for H₂S in adipose tissue metabolism appears to be its role in reducing inflammatory cytokine production from resident adipose macrophages. These cytokines are a known causal factor in the development of insulin resistance in adipose and other metabolic tissues [34]. In one study, macrophages isolated from mice with diet-induced obesity produced less H₂S and more cytokines than macrophages from lean mice [5]. Based on these reports, it may be important that future work in adipose tissue (from obese subjects) separates the adipocytes from the stromal vascular fraction. Several studies have also shown a role for the H₂S/CSE system in perivascular adipose tissue, although
most of this work has described its importance in vascular tone (reviewed in [35]) rather than glucose homeostasis.

Another key organ in the regulation of glucose metabolism is the liver. During an elevated circulating glucose scenario, insulin acts on the liver to stimulate glucose uptake and its conversion to glycogen and fatty acids for storage. In a low glucose scenario, pancreatic glucagon acts on the liver to promote the production or liberation of glucose through gluconeogenesis or glycogenolysis, respectively. Dysregulation of insulin signaling in the liver (hepatic insulin resistance) is a common phenomenon in T2DM (reviewed in [36]). The mRNA expression of both CSE and CBS was demonstrated in the liver of rats and was found to increase after inducing type 1 diabetes with STZ [4]. Later on it was demonstrated that overexpressing CSE in hepatocytes leads to reduced type 2 diabetes with STZ [4]. Later on it was demonstrated that overexpressing CSE in hepatocytes leads to reduced glycogen content. In this study, it was also shown that CSE KO animals (lower H₂S) have a reduction in endogenous glucose production [37]. A recent study by Ju and colleagues demonstrated a mechanism by which H₂S may directly stimulate glucoseogenesis. They found that pyruvate carboxylase (a key enzyme in gluconeogenesis) is sulfhydrated by H₂S, which leads to increased activity and glucose production [9]. These findings seem to indicate that H₂S production in the liver causes enhanced glucose release, an effect that could aggravate the hyperglycemia observed in diabetes. However, since type 2 diabetes are known to have lower rather than higher circulating H₂S, further studies investigating the liver production of H₂S during T2DM are required.

Surprisingly, there is a paucity of studies that have examined the role of H₂S in skeletal muscle, let alone skeletal muscle glucose uptake. This may be due in part to the low or nondetectable levels of the H₂S producing enzymes in rodent models (in contrast to the higher levels found in human muscle, reviewed in [38]). Nevertheless, future work should, at the very least, examine the effects of H₂S donors since H₂S may act on muscle tissue via its circulating stores.

7. Other Hormones and Future Work

While H₂S plays important roles in the metabolism of hormones like insulin and glucagon, a variety of other metabolic hormones remain to be examined. One emerging area holding potential for this is the gastrointestinal endocrine system. Here, a variety of enteroendocrine cells secrete numerous peptide hormones that play important roles in glucose homeostasis and energy metabolism. Some important candidates are the insulin-stimulating incretin hormones: glucose-dependent insulinotropic polypeptide (GIP) and glucagon peptide-1 (GLP-1). Recently, Bala and colleagues examined the role of endogenous H₂S in a GI endocrine cell line, STC-1 [39]. This cell line secretes GLP-1 and the anorexic hormone peptide YY (PYY). They found that H₂S donors and l-cysteine impaired oleic acid-stimulated GLP-1 and PYY secretion. While their primary focus was on the modulatory effect of H₂S on oleic acid-stimulated hormone secretion, their results support further investigation of H₂S on GI hormone secretion and signaling. Indeed, the question remains: can GI endocrine cells produce their own H₂S, and is the altered H₂S level observed in obesity responsible for the dysregulation in GI hormone secretion [40]? Of importance, GLP-1 therapies have become a major tool in the treatment of type 2 diabetes [41] and recently obesity [42]. Therefore, the role H₂S has in GLP-1 and other endocrine cells may be an additional mechanism by which this gasotransmitter can regulate glucose homeostasis.

Competing Interests

The authors declare that they have no competing interests.

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