

FOLIA MEDICA CRACOVIENSIA

Vol. LVII, 1, 2017: 55–64

PL ISSN 0015-5616

Nicotine affects hydrogen sulfide concentrations in mouse kidney and heart but not in brain and liver tissues

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Abstract: Nicotine, a potent parasympathomimetic alkaloid with stimulant effects, is contributing to addictive properties of tobacco smoking and is though used in the smoking cessation therapy. Hydrogen sulfide (H₂S) is involved in physiology and pathophysiology of various systems in mammals. The interactions between nicotine and H₂S are not fully recognized. The aim of the study is to assess the influence of nicotine on the H₂S tissue concentrations in different mouse organs. Adult CBA male mice were administered intraperitoneally 1.5 mg/kg b.w. per day of nicotine (group D1, n = 10) or 3 mg/kg b.w. per day of nicotine (group D2, n = 10). The control group (n = 10) received physiological saline. The measurements of the free and acid-labile H₂S tissue concentrations were performed with the Siegel spectrophotometric modified method. There was a significant increase in H₂S concentrations in both nicotine doses groups in the kidney (D1 by 54.2%, D2 by 40.0%). In the heart the higher nicotine dose caused a marked decrease in H₂S tissue level (by 65.4%), while the lower dose did not affect H₂S content. Nicotine administration had no effect on H₂S concentrations in the brain and liver.

In conclusion, nicotine affects H₂S tissue concentrations in kidney and heart but not in the liver and brain tissues.

Key words: nicotine, hydrogen sulfide, brain, heart, kidney, liver, mouse.

Introduction

Nicotine is a potent parasympathomimetic alkaloid found in the nightshade family of plants including *Nicotiana tabacum*. It serves as an agonist towards different subtypes of five-subunit nicotinic acetylcholine receptors (nAChR), members of the ‘Cys-loop’ superfamily, whereas in specific subtypes of them — nAChR α 9 and nAChR α 10 it acts as an antagonist [1]. Nicotinic receptors play diverse and often critical functions throughout the central and peripheral nervous systems and have emerging roles in non-neuronal systems. The nAChRs have been identified i.a. in bronchial epithelial cells, endothelial cells, vascular smooth muscle cells, mesangial cells, inflammatory cells and keratinocytes [2, 3].

Nicotine has stimulant effects and is contributing to addictive properties of tobacco use and is therefore widely applied in the smoking cessation therapy [4]. Cigarette smoking has great socioeconomic repercussions — it is the leading cause of preventable mortality and disability worldwide — the habit affects one billion people throughout the world and causes near six million deaths each year [5]. Furthermore, nicotine administration poses several health hazards. The alkaloid has been demonstrated to affect cell proliferation, oxidative stress, apoptosis, decrease immune response and contribute to DNA damage what altogether leads in effect to increased risk of cardiovascular events, respiratory, reproductive and gastrointestinal disorders and cancer development [6, 7].

The studies of the recent decade have uncovered the biological significance of hydrogen sulfide (H₂S) and subsequently changed its perception from an odorous by-product of putrescence processes to a crucial co-modulator in physiology and pathophysiology of various systems in mammals [8]. H₂S is formed in a series of enzymatic reactions in which the substrates are sulfur-containing amino acids such as L-cysteine, L-homocysteine and D-cysteine. The key enzymes are cystathionine γ -lyase (CSE), cystathionine β -synthase (CBS), cysteine aminotransferase (CAT), 3-mercaptopyruvate sulfur transferase (3-MST) and D-amino acid oxidase (DAO). H₂S is also a product in a few other enzymatic reactions of the complex sulfur metabolism and in non-enzymatic reactions [9]. It is recognized that H₂S acts mainly in a paracrine manner but it is also transported to other tissues as a component of the serum and inside erythrocytes. This gaseous messenger exerts a broad array of biological actions. No specific receptor to H₂S has been identified. The molecular

targets of H₂S are varied and comprise many proteins which participate in the regulation of crucial physiological processes. The most important mechanisms of action involve sulfhydration of proteins and their redox regulation, modification of prosthetic groups, interaction with nitric oxide (NO) and carbon monoxide (CO), participation in S-nitrosylation of proteins, interaction with reactive oxygen species (ROS) and electrophile lipid derivatives [10]. The biological effects of H₂S cover changes in the activity of ion channels, N-methyl-D-aspartate (NMDA) receptors, various signaling proteins, transcription factors, enzymes, structural and transport proteins [11].

Data on the share of H₂S in the development of complications of smoking are scarce. H₂S alleviated airway reactivity induced by acetylcholine (ACh) or potassium chloride and reduced inflammatory cytokines interleukin 8 and tumor necrosis factor α (TNF-α) in a rat model of chronic exposure to cigarette smoke [12]. The mediator ameliorated tobacco smoke-induced oxidative stress and emphysema in mice and pulmonary fibrosis in smoking rats via attenuation of oxidative stress and inflammation [13, 14]. H₂S improved left ventricular function in smoking rats via regulation of apoptosis and autophagy [15]. Additionally, mounting evidence shows pivotal role of H₂S in the function of the brain and changes of H₂S generation in cases of exposure to different compounds and drugs [9, 16, 17].

The interactions between nicotine, cigarette toxins and H₂S are not fully recognized [18]. The indication of the influence of nicotine on sulfur metabolism might help to evolve more efficient smoking cessation and addiction therapies. Moreover, the elaboration of compounds correcting sulfur biology disturbances observed in various smoking-related diseases might hamper their progression and improve mortality and morbidity outcomes.

The aim of the study is to assess the influence of nicotine on the endogenous H₂S tissue concentrations in different mouse organs.

Material and methods

Animals

The study has been approved by the II Local Ethics Committee for Animal Experimentation in Krakow at the Polish Academy of Sciences (Krakow, Poland) with the permission no. 24/2016 issued on 22nd March 2016.

Thirty CBA male mice (15-week-old individuals) of approximate 20 g weight were involved in the study. The animals were housed under standard laboratory conditions and had free access to water and food. They were kept at temperature of 22–24°C with a light/dark cycle of 12 h (8 am — 8 pm and 8 pm — 8 am, respectively).

Study design

With the median lethal dose estimated at around 4 kg per kg body weight in mice, the study protocol comprised intraperitoneal injections of nicotine dissolved in a saline solution in doses of 1.5 mg per kg of body weight daily (group D1, $n = 10$) or 3 mg per kg of body weight daily (group D2, $n = 10$) for 5 consecutive days at the same time of the day (9:00 am) — each administration of 0.2 ml [19]. The control group ($n = 10$) received intraperitoneally physiological saline in portions of the same volume. The individuals were randomly assigned to each group. The animals tolerated the applied doses of nicotine well and remained in good condition till the end of the experiment. Measurements of the free and acid-labile tissue H_2S concentrations were performed by the use of the modified method of Siegel [20, 21]. A standard curve was prepared with sodium sulfide solutions simultaneously analyzed by the iodometric titration.

Tissue sample preparation

An hour after the last nicotine solution or physiological saline injections the animals were killed by cervical dislocation. Brain, heart, kidney and liver tissues of each animal were quickly removed, homogenized with 0.01 M sodium hydroxide (NaOH) and frozen. Each tissue was combined with NaOH in different proportions (brain: 1 to 4, kidney and liver: 1 to 5 and heart: 1 to 10). Then, 50% trichloroacetic acid (TCA) was added to the samples. The TCA solution (0.5 ml) was added to 2 g of brain or liver samples in tight 3 ml capsules, and 0.25 ml was added to 1 g of heart or kidney sample in tight 2 ml capsules. These suspensions were shaken, and the resultant mixture was centrifuged. Subsequently, 1.5 ml brain or liver and 0.75 ml heart or kidney supernatant samples were moved to 2 ml tight capsules with 0.15 ml or 0.075 ml of 0.02 M N,N-dimethyl-p-phenylenediamine sulfate in 7.2 M hydrochloric acid (HCl), and 0.15 ml or 0.075 ml of 0.03 M iron (III) chloride ($FeCl_3$), respectively, was then added in 1.2 M HCl portions. After 20 min in the dark, the contents were shaken for 1 min with 1 ml of chloroform.

H_2S tissue concentration measurements

The absorbance was measured at 650 nm with a Varian Cary 100 spectrophotometer. A standard curve was prepared with an iodometrically determined 0.0001 M sodium sulfide (Na_2S) solution. Four concurrent analyses of every analyzed tissue type were performed for each group of animals.

Statistical analysis

The statistical analysis was performed with the Statistica 10 PL version (Statsoft, Tulsa, USA). Normal distribution of variables was disproved using the Shapiro-Wilk test. Subsequently, the existence of differences in H₂S tissue concentrations among groups within one organ were assessed with Kruskal–Wallis one-way analysis of variance. The Mann-Whitney U test was used in the post hoc analysis for comparisons between selected pairs of groups (control group vs D1 group and control group vs D2 group). Statistical significance was considered when $p < 0.05$.

Results

There was a significant increase in H₂S concentrations in both nicotine doses groups in the kidney (D1 by 54.2%, D2 by 40.0%). In the heart the higher nicotine dose caused a marked decrease in H₂S tissue level (by 65.4%), whereas the lower one did not affect H₂S content (control group vs D2 group: 8.45 ± 0.07 µg/g vs 8.15 ± 0.13 µg/g, $p = 0.1143$). Nicotine administration had no effect on H₂S concentrations in the brain and liver. H₂S tissue levels' results are presented in the Table 1.

Table 1. Hydrogen sulfide (H₂S) tissue concentration in mouse brain, heart, liver and kidney following the administration of 1.5 mg/kg b.w. per day or 3 mg/kg b.w. per day of nicotine (groups D1 and D2, respectively).

H ₂ S tissue concentration [µg/g]	Control group (n = 10)	D1 (n = 10)	D2 (n = 10)	Kruskal-Wallis one-way analysis of variance	Control vs D1 – Mann-Whitney U test	Control vs D2 – Mann-Whitney U test
Brain	1.70 ± 0.06	1.74 ± 0.03	1.81 ± 0.07	NS	NS	NS
Heart	8.45 ± 0.07	8.15 ± 0.13	2.92 ± 0.12	H(2, 12) = 8.80 p = 0.0123	NS	p = 0.0294
Liver	2.43 ± 0.06	2.73 ± 0.10	2.46 ± 0.08	NS	NS	NS
Kidney	4.48 ± 0.17	6.91 ± 0.21	6.27 ± 0.16	H(2, 12) = 9.85 p = 0.0073	p = 0.0286	p = 0.0303

NS — statistically not significant

Discussion

In our experiment nicotine administration caused H₂S tissue content elevation in the kidneys. Nicotine has worsened kidney function in different studies on animal and human acute and chronic renal failure. The alkaloid was shown to be a powerful stimulus for human mesangial cell proliferation and fibronectin production. These effects were accompanied by the increased activity of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase, leading to ROS and nitrotyrosine production, mitogen-activated protein kinases (MAPKs) activation, and were prevented by protein kinase C (PKC) inhibition [2, 22, 23]. Glomerular injury induced by nicotine was also shown to be mediated by augmented cyclooxygenase-2 (COX-2) expression and activity what was partially triggered by ROS [24]. Interestingly, NADPH oxidase, MAPKs, PKC are COX-2 are among molecular targets of H₂S and H₂S exerts a broad array of protective actions in kidney failure conditions [10, 25]. H₂S was demonstrated to inhibit nicotine and lipopolysaccharide-induced osteoclastic differentiation by blocking the activation of MAPKs, phosphoinositide 3-kinase (PI3K), PKC and nuclear factor-κB (NF-κB) [26]. Plasma concentration of H₂S correlated negatively with the levels of conventional PKC βII activation and cardiovascular mortality index in chronic hemodialysis patients [27]. H₂S administration in uranium-intoxicated rats inhibited uranium-induced nuclear translocation and phosphorylation of NF-κB-p65, which decreased protein expression of target-p65 inflammatory genes of TNF-α, inducible nitric oxide synthase (iNOS) and COX-2 [28]. H₂S quenched angiotensin II-induced NADPH expression in cardiac fibroblasts what was associated with decreased ROS production, reduced MAPKs (Erk 1/2) phosphorylation and connective tissue growth factor (CTGF) expression [29].

In contrast, there are studies demonstrating anti-inflammatory effects of nicotine occurring under specific circumstances. Single injections of the alkaloid attenuated the severity of renal injury in rat models of ischemia-reperfusion and in a model of sepsis which was associated with reductions in inflammatory cytokines and NF-κB, what is observed among numerous biological actions of H₂S [30–32]. Additionally, long-term administration of nicotine in the drinking water resulted in improvements in proteinuria and renal function in a rat model of proteinuria-induced renal inflammation with unclear mechanisms [33]. These protective effects of nicotine might be partially mediated by H₂S.

As we have shown, nicotine caused decrease in H₂S levels in the heart. Nicotine was proven to dysregulate sympathovagal balance in the heart, affecting heart rate, blood pressure, myocardial contractility, increasing myocardial demand for oxygen and nutrients, causing vasoconstriction and promoting tissue remodeling and fibrosis [3, 34]. In the study by Ji and colleagues nicotine administration to isolated perfused rat hearts caused a brief decrease followed by a much larger increase in the heart

rate [35]. The effects were attributed by the authors to different nAChRs subtypes share but interactions with mediators in autonomic nervous system including H₂S should strongly be considered. H₂S was shown to modulate fast cholinergic synaptic input acting on presynaptic terminals in peripheral ganglia and neuromuscular junction activity what involved presynaptic ryanodine receptor [36, 37]. Furthermore, H₂S was considered as an endothelium-derived hyperpolarizing factor mediated by Ach in rat cerebral vessels [38].

It should be emphasized that nicotine is only one of more than 9000 compounds found in cigarette smoke. This smoke contains many oxidizing chemicals, CO, volatile organic compounds, particulates, heavy metals, all with more than 60 identified carcinogens [39, 40]. The effects of nicotine are accompanied by many other actions of cigarette smoke contents.

Growing evidence has revealed that H₂S improves heart function and reduces cardiac complications in different pathogenic conditions. H₂S exerts antioxidative actions, preserves mitochondrial function, has anti-inflammatory and antiapoptotic properties, angiogenic effects on the ischemic area, participates in the regulation of ion channels and microRNAs (miRNAs) expression, and interferes with NO and CO [41]. Thus, our findings of nicotine reducing H₂S content in the heart stresses the need for new directions of study on possible exogenous H₂S supplementation in smoking and nicotine replacement therapy.

We have not observed any effect of nicotine administration on H₂S tissue levels in the brain and liver. In tobacco smoking the addictive nature of nicotine involves psychoactive effects, drug-reinforced behavior, compulsive use, relapse after abstinence, physical dependence and tolerance [42]. These complex actions are mediated by close interactions of the glutamatergic, dopaminergic and γ -aminobutyric acid systems mainly in the mesocorticolimbic system [5]. H₂S is perceived as a potent neuroprotector and neuromodulator [9].

In the liver nicotine evoked organ damage inducing oxidative stress, biochemical changes and histopathological injuries [43–45]. To the contrary, H₂S displayed a crucial role in physiology and protective actions in liver pathology [46].

In conclusion, the influence of nicotine on H₂S tissue concentrations in the kidney and heart tissues proves that the biological actions of nicotine involve impact on sulfur metabolism of undetermined mechanisms. Interestingly, the interactions between H₂S and nicotine seem to begin long before nicotine is inhaled with cigarette smoke or administered as replacement therapy in smoking cessation: H₂S has been recently identified as a crucial modulator of nicotine biosynthesis in *Nicotiana tabacum* [47]. Our results are highly indicative but less conclusive. They open new fields of research for the use of H₂S donors and HS-releasing agents in the prevention of the complications of smoking and nicotine replacement therapy. Neither have we examined the influence of long-term exposure to nicotine, nor the effects of other

cigarette smoke constituents, what poses our experiment's limitations. No effect of nicotine on H₂S levels in the liver and brain absolutely does not preclude their interactions, especially in chronic exposure to nicotine.

Acknowledgments, funding, and disclosures

This study has not been funded.

Conflict of interest

None declared.

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