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Allyl disulfide as donor and cyanide as acceptor of sulfane sulfur in the mouse tissues

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Abstract:
Cytoplasm of mammalian glial cells was reported to contain Gomori-positive cytoplasmic granulation (GPCG), whose biological role is unknown. The present study attempted to discover conditions facilitating GPCG formation and to elucidate their relationship with sulfane sulfur metabolism. To address these problems, we investigated *in vivo* the effect of both allyl disulfide (DADS), occurring in garlic (sulfane sulfur donor) and cyanide (sulfane sulfur acceptor) on number of GPCG-containing glial cells in the mouse brain. In parallel, sulfane sulfur level and activity of rhodanese and 3-mercaptopyruvate sulfurtransferase (MpST) were determined in the mouse brain and liver. Cyanide caused a drop in GPCG number in the brain, while activity of sulfurtransferases and sulfane sulfur level remained unchanged. Slight but significant cyanide-induced rise in MpST activity was observed only in the liver, which indicates a possibility of enhancement of its detoxification in reaction with mercapropyruvate in this organ. DADS, a sulfur donor, increased GPCG number in the brain, whereas activity of sulfurtransferases and sulfane sulfur level did not change. However, in the liver, DADS elevated both sulfurtransferase activity and sulfane sulfur level.
These observations suggest that DADS can constitute a source of sulfane sulfur for the liver, thereby activating anaerobic sulfur metabolism and sulfane sulfur transfer. Consequently, this leads to the increase in sulfane sulfur level in plasma, in which it is transported in the form of albumin hydropersulfides and can be used for cyanide detoxification or stored in glial cells as GPCG. Therefore, it is not excluded that GPCG observed in the brain of mice and other mammals can be a source and a store of sulfane sulfur in mammals.

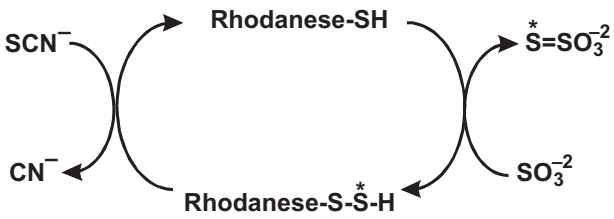
Key words:
allyl disulfide, cyanide, Gomori-positive cytoplasmic granulation in glial cells of mammalian brain

Abbreviations: DADS – allyl disulfide, GPCG – Gomori-positive cytoplasmic granulation; MpST – 3-mercaptopyruvate sulfurtransferase; ROS – reactive oxygen species

Introduction

Compounds containing sulfane sulfur exhibit *in vitro* regulatory and antioxidant properties [5, 9, 15, 24], but they cannot be applied in biological systems, because they are unstable and are quickly decomposed at physiological pH.

Sulfane sulfur-containing compounds are formed in anaerobic cysteine metabolism and are characterized by the presence of exceptionally reactive sulfur atom, occurring in the 0 or –1 oxidation state, which is covalently bound to another sulfur atom (R-S-S*-H). Sulfur with such properties easily leaves structure of the compound, and can be transferred to different acceptors, like sulfates (IV) (SO_3^{2-}) or cyanide (CN^-). For the latter acceptor, it is often called "cyanolysable sul-



Scheme 1. Transfer of sulfur atom from thiosulfate to cyanide with rhodanese participation

fur". External sulfur atom of thiosulfate (S* = SO_3^{2-}) and elemental sulfur (S_8) have also properties of sulfane sulfur (Scheme 1). Sulfane sulfur plays an important role not only in cyanide detoxification, but also it participates in the formation of iron-sulfur proteins. Hydropersulfides (RSSH), such as thiocysteine, are more efficient hydrogen donors in comparison with sulfhydryl group, and being persulfide anions (RSS^-), they are more reactive electron donors, which makes them remarkably effective antioxidants [5].

Sulfane sulfur fulfills regulatory function in the cells by converting protein –SH groups to persulfides or trisulfides [5, 23] since in this way enzymatic and receptor proteins can be inhibited or bioactivated [9, 26].

Disulfides, containing double bond or carbonyl group in their structure (R-S-S-CH_2-CH=CH-R; R-S-S-CH_2-CO-CO_2H and R-S-S-CH_2-COH) are another class of compounds characterized by the presence of the highly reactive labile sulfur. Such structure makes the bonds between carbon and sulfur more labile which enables their tautomerization to thiosulfoxides, and allyl disulfide is a good example of such compounds [24]. For this reason, these compounds attract attention as potential exogenous source of labile sulfane sulfur [8].

In acute poisoning, cyanide is a quickly acting poison of the respiratory system, due to its ability to inhibit cytochrome oxidase [11], whereas chronic cyanide intoxications, caused by progressive industrialization and cyanogenic substances present in food products, lead to neurological disorders [28]. There are reports indicating that neurotoxic cyanide effect can be related to reactive oxygen species (ROS) generation [30], nitric oxide biosynthesis [31] and redox-dependent activity of transcription factor $NF_\kappa B$ [16]. Toxicity of CN^- can also be connected with induction of cyclooxygenase-2 (COX-2) in nervous cells [11] or inhibition of activity of antioxidant enzymes, such as catalase and superoxide dismutase (SOD) [10]. Furthermore, final effect of cyanide toxicity is determined by competitive reactions of its detoxification to SCN^-, catalyzed by rhodanese [EC 2.8.1.1] and 3-mercaptopyruvate sulfurtransferase (MpST) [EC 2.8.1.2] [3]. Cyanide detoxification by rhodanese requires the presence of compounds bearing labile sulfane sulfur, while mercaptopyruvate, MpST substrate, is needed for detoxification by the latter enzyme.

Histopathological studies of the mammalian brain have demonstrated that glial cells contain GPCG rich in reduced sulfur, whose biological role is unknown [21]. The studies of Srebro and Lach [20] suggest possibility of their participation in reactions, shielding the cells from peroxidative damage. The aim of the present study was an attempt to elucidate a biological role of GPCG and to ascertain conditions of their formation and connections with sulfane sulfur metabolism. In order to achieve this goal, we conducted *in vivo* studies on the effect of both allyl disulfide, sulfane sulfur "donor" and cyanide, sulfane sulfur "acceptor" on number of GPCG in glial cells from the mouse brains. In addition, sulfane sulfur level and activities of rhodanese and 3-mercaptopyruvate sulfurtransferase were determined in the liver and brain of the animals.

Materials and Methods

Animals

Female albino Swiss mice (*Mus musculus* L.) weighing approximately 20 g were used. In the course of the experiment, the animals were kept under standard laboratory conditions and were fed a standard chow. All procedures were approved by the Ethics Committee for the Animal Research in Kraków (nr 74/OP/2002).

The mice were divided into 4 groups, containing 10 animals each. The experimental animals in the first group were treated *ip* with potassium cyanide (KCN) at 0.4 mg/kg for four days. The control group for these mice was injected *ip* with 0.9% NaCl (second group). The animals in the third group were treated with allyl disulfide (DADS) at 50 mg/kg dissolved in corn oil, for ten days. The control mice were injected with the same volume of the corn oil (fourth group).

All the mice were sacrificed by cervical dislocation. Immediately thereafter, the brains from five animals were quickly removed, trimmed, fixed by immersion in Bouin's fluid and subjected to the histological experiments. Paraffin-embedded material was cut serially into 7 μm sections and stained with Gomori's chrome hematoxylin-phloxin method with Bargmann's modification. Glial cells containing GPGC rich in sulfur were counted in periventricular brain tissue around the anterior part of the third ventricle. The area, in which the GPGC were counted was 0.1125 sq mm large. The cells were counted in such areas in identical places in adjacent sections of the brains of all experimental and control animals.

All livers and the next five brains were quickly excised from animals of all groups, frozen and kept at –76°C until used in biochemical experiments. Thereafter, the frozen livers and brains were weighed and homogenized, 1 g of the tissue in 4 ml of 0.1 M phosphate buffer, pH 7.4. Brain and liver homogenates were next used for assay of sulfane sulfur level and activity of sulfurtransferases.

Chemicals

Potassium cyanide (KCN), dithiothreitol, p-phenylenodiamine, N-ethylmaleimide (NEM), β-nicotinamide adenine dinucleotide reduced form (NADH) and lactic dehydrogenase (LDH) were provided by Sigma Chemical Co. (St. Louis, MO, USA). Allyl disulfide (DADS) was purchased from Fluka Chemie AG (Switzerland), Buchs**.** Ammonium 3-mercaptopyruvate was synthesized according to Sprison and Chargaff [19]. Formaldehyde, ferric chloride ($FeCl_3$), thiosulfate, sodium sulfite were obtained from the Polish Chemical Reagent Company (P. O. Ch. Gliwice, Poland).

Determination of sulfane sulfur level

The level of the compounds containing sulfane sulfur was determined by the method of Wood [29] based on cold cyanolysis and colorimetric detection of ferric thiocyanate complex ion.

Determination of bound sulfane sulfur level

This pool of sulfane sulfur was assayed by the modification of Ogasawara's method [13]. In this method, bound sulfur is easily liberated as sulfide after reduction by dithiothreitol. The released sulfide is converted into a fluorescent derivative, thionine, through the reaction with p-phenylenediamine and ferric ion. Thionine is determined using fluorometric detection.

Determination of rhodanese activity

The activity of rhodanese was assayed according to Sorbo's method [18] measuring the amount of SCN^- formed during the 5 min of incubation at 20°C.

Determination of 3-mercaptopyruvate sulfurtransferase (MpST) activity

The activity of MpST was determined by measuring the amount of the pyruvate formed during 15 min of incubation at 37°C in accordance with the method of Valentine and Frankenfeld [25].

Statistical analysis

The mean and standard deviation of the mean were calculated for each group. The statistical significance of the differences was evaluated using analysis of variance and one-way ANOVA test.

Results

Results of histological studies (Fig. 1) demonstrated that number of glial cells containing GPCG in the hypothalamus of control animals (0.9% NaCl) averaged

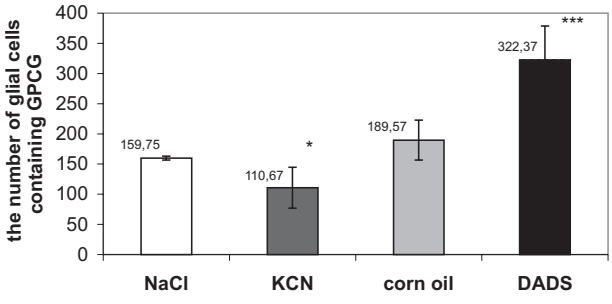


Fig. 1. The effect of intraperitoneal administration of potassium cyanide (KCN) (0.4 mg/kg of body weight) and allyl disulfide (DADS) (50 mg/kg) on number of glial cells containing Gomori-positive cytoplasmic granulations (GPCG) rich in sulfur in the hypothalamus of the mouse brain. * statistically significant difference between control and experimental animals ($p \leq 0.05$). *** statistically significant difference between control and experimental animals. ($p \leq 0.001$)

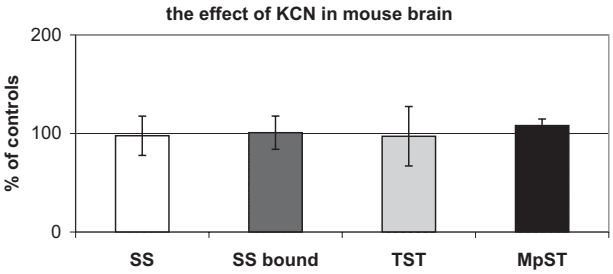


Fig. 2. The effect of intraperitoneal administration of potassium cyanide (KCN) at a dose of 0.4 mg/kg for 4 days on the level of sulfane sulfur (SS), bound sulfane sulfur (bound SS) and on activity of rhodanese (TST) and 3-mercaptopyruvate sulfurtransferase (MpST) in the mouse brain. Values are expressed as a percent of the control. The control values are as follows: SS – 102.75 ± 14.54 nmoles g^{-1} wet weight; bound SS – 110.04 ± 9.03 nmoles g^{-1} wet weight; TST – 19.39 ± 3.40 μmoles $g^{-1}min^{-1}$; MpST: 72.19 ± 10.08 μmoles $g^{-1}min^{-1}$

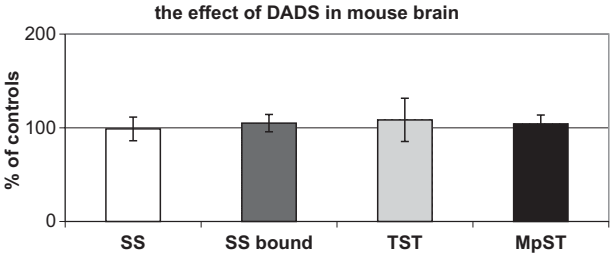


Fig. 3. The effect of intraperitoneal administration of allyl disulfide (DADS) at a dose 50 mg/kg for 10 days on the level of sulfane sulfur (SS), bound sulfane sulfur (bound SS) and on activity of rhodanese (TST) and 3-mercaptopyruvate sulfurtransferase (MpST) in the mouse brain. Values are expressed as a percent of the control. The control values are as follows: SS – 103.00 ± 19.40 nmoles g^{-1} wet weight; bound SS – 68.21 ± 7.37 nmoles g^{-1} wet weight; TST – 17.58 ± 3.09 μmoles g^{-1} min^{-1}; MpST – 84.21 ± 8.11 μmoles g^{-1} min^{-1}

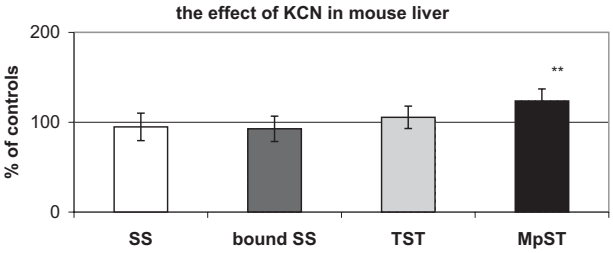


Fig. 4. The effect of intraperitoneal administration of potassium cyanide (KCN) at a dose of 0.4 mg/kg for 4 days on the level of sulfane sulfur (SS), bound sulfane sulfur (bound SS) and on activity of rhodanese (TST) and 3-mercaptopyruvate sulfurtransferase (MpST) in the mouse liver. Values are expressed as a percent of the control. The control values are as follows: SS: 707.19 ± 60.45 nmoles g^{-1} wet weight; bound SS – 96.63 ± 9.20 nmoles g^{-1} wet weight; TST – 172.69 ± 16.21 μmoles g^{-1} min^{-1}; MpST – 594.21 ± 70.39 μmoles g^{-1} min^{-1}. ** statistically significant difference between control and experimental animals ($p \leq 0.01$)

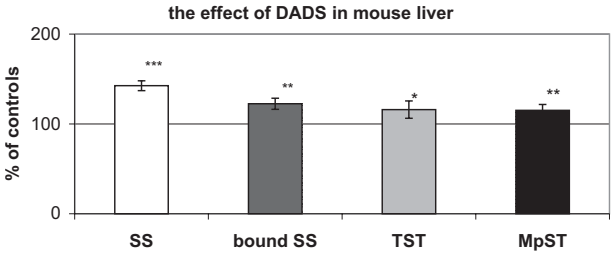


Fig. 5. The effect of intraperitoneal administration of allyl disulfide (DADS) at a dose of 50 mg/kg for 10 days on the level of sulfane sulfur (SS), bound sulfane sulfur (bound SS) and on activity of rhodanese (TST) and 3-mercaptopyruvate sulfurtransferase (MpST) in the mouse liver. Values are expressed as a percent of the control. The control values are as follows: SS – 543.17 ± 29.31 nmoles g^{-1} wet weight; bound SS – 107.62 ± 7.54 nmoles g^{-1} wet weight; TST – 115.32 ± 8.58 moles g^{-1} min^{-1}; MpST – 391.67 ± 21.88 μmoles g^{-1} min^{-1}. * statistically significant difference between control and experimental animals ($p \leq 0.05$). ** statistically significant difference between control and experimental animals ($p \leq 0.01$). **** statistically significant difference between control and experimental animals ($p \leq 0.001$)

159.75 for the second group (0.9% NaCl) and 189.57 for the fourth group (corn oil). In the experimental group of mice which were administered *ip* with KCN solution, there was a statistically significant drop in GPCG number (69.28%). DADS injection caused high significant increase in GPCG number above the control level (170.05%).

Parallel studies indicated that cyanide treatment (at a dose of 0.4 mg/kg for 4 days) did not cause any changes in sulfane sulfur level (either total or bound) or rhodanese activity in the mice liver and brain. Cyanide induced only a rise in MpST activity in the liver (Fig. 4), while its level in the brain remained unchanged (Fig. 2).

Intraperitoneal administration of DADS, which is a sulfane sulfur "donor", did not lead to any observable alterations in sulfane sulfur level or activity of both sulfurtransferases in the animal brains (Fig. 3). On the other hand, DADS elevated rhodanese and MpST activity and both "total" and "bound" sulfane sulfur level in the liver (Fig. 5).

Discussion

The role of GPCG occurring in the brain and conditions of their formation are completely unknown. A hypothesis has been proposed that anaerobic cys-

teine sulfur metabolism can be the source of these formations (Scheme 1). For this reason, a relationship between sulfane sulfur level in mouse tissues and GPCG number in the brain glial cells was investigated.

The studies showed that DADS as well as cyanide changed the number of GPCG in the mouse brain. It was accompanied by an increase in activity of sulfurtransferases and sulfane sulfur level in the liver after DADS treatment, while cyanide intoxication altered neither activity of sulfurtransferases nor sulfane sulfur level in the mouse liver and brain. Slight but statistically significant increase in MpST activity induced by cyanide was observed only in the liver, which indicates that CN^- detoxification in the reaction with mercaptopyruvate takes place in the cytosol. This means that cyanide does not change the level of rhodanese substrates (i.e. sulfane sulfur-containing compounds) in these organs or activity of sulfurtransferases participating in CN^- detoxification. Therefore, the lack of influence of CN^- on tissue sulfane sulfur level and activity of sulfurtransferases, observed in our studies, suggests that under these conditions, another mechanism responsible for cyanide detoxification can be triggered. Since simultaneously CN^- induces statistically significant drop in GPCG number in the brain, it can be suggested that cyanide can be detoxified in blood, and that sulfur released from Gomori-positive cytoplasmic granulation in the brain glial cells can participate in this process. This suggestion appears to be corroborated by results of *in vivo* studies of Buzaleh et al. [1, 2]. These authors observed that upon chronic cyanide treatment, sulfane sulfur level and rhodanese activity were elevated only in blood and in the heart. This implies that during cyanide treatment, sulfane sulfur is released into blood, that underlines particular role of blood in cyanide detoxification and preventing it from reaching the tissues. The results presented in this paper also show that CN^- does not influence rhodanese activity and sulfane sulfur level in the mouse liver and brain which suggests that cyanide is not detoxified in these organs.

In vivo studies into cyanide toxicity have demonstrated that its toxic effects depend on CN^- dose, time and route of its administration and on animal species. Main pathway of biological CN^- detoxification involves rhodanese, which requires sulfane sulfur as a substrate [27]. SCN^-, which is easily excreted by the kidneys, is the end product of this reaction. Critical importance of sulfane sulfur for reactions of cyanide detoxification is substantiated by interrelationship between rhodanese, an enzyme participating in sulfane sulfur transfer and γ-cystathionase (γ-CT), an enzyme involved in sulfane sulfur biosynthesis (Scheme 1) [22]. It has been noted that inhibition of γ-CT activity leads to an increase in CN^- toxicity [14]. Deficit of sulfane sulfur, a rhodanese substrate (Scheme 1), disables formation of hydropersulfide in its active center, which is a prerequisite for sulfur transfer to cyanide and SCN^- formation [7].

The liver and brain are characterized by high activity of rhodanese and MpST, and by capability of biosynthesis of sulfane sulfur and mercaptopyruvate [3], which affords them efficient protection against CN^- toxicity [12]. On the other hand, the brain, heart and lungs, where activity of sulfurtransferases and enzymes involved in sulfane sulfur biosynthesis is low or negligible, are much more endangered by toxic action of cyanide. Therefore, cyanide detoxification in blood, with the use of labile sulfane sulfur released from GPCG may be crucial for these organs. Hence, results of the present studies suggest contribution of these granulations to cyanide detoxification processes. Investigations conducted by Srebro and Lach [20] demonstrated that X-ray and UV irradiation could raise GPCG number and also suggested protective role of GPCG caused by their involvement in reduction of organic hydropersulfides.

The present results showed that an increase in GPCG number could also be evoked by DADS, a sulfane sulfur "donor", which was also accompanied by elevated activity of sulfurtransferases in the liver but not in the brain (!). This means that this compound can be a source of sulfane sulfur for the liver, activating anaerobic sulfur metabolism and sulfane sulfur transfer. Consequently, DADS treatment led to augmentation of sulfane sulfur level in plasma, where it is transported in the form of albumin hydropersulfides [24], and then probably stored in glial cells of the mouse brain. Therefore, the presence of granulations rich in reduced sulfur in the brains of mice and other mammals can be a way of storage of reactive, labile sulfur and then its source for the reactions of cyanide detoxifications in the circulation.

Cyanide neurotoxicity results from inhibition of cytochrome oxidase of the respiratory chain and other metalloproteins, and is associated with the presence of carbonyl groups [17] and accelerated ROS generation, which leads to peroxidative damage [11]. Therefore, toxic action of CN^- can be prevented by both antioxidants, like N-acetylcysteine or trolox and sulfane

sulfur-containing compounds. DADS and other compounds of this type can perform both these functions simultaneously, since they contain reactive sulfane sulfur which facilitates CN^- detoxification to SCN^- as well as possesses antioxidant action [5, 6].

Conclusions

The presented studies demonstrate that DADS a sulfane sulfur donor increases GPCG number in glial cells of the mouse brain, while cyanide, a sulfane sulfur acceptor lowers it. This indicates that: (1) sulfur contained in granulations in glial cells can be released to the circulation, where it is used for cyanide detoxification; (2) formation of GPCG may be a store of sulfane sulfur that can be replenished from exogenous donors, such as DADS occurring in garlic.

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