Methane biogenesis during sodium azide-induced chemical hypoxia in rats

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Our primary objective in the present investigation was to identify mechanistic details of the methanogenic reactions in a whole animal model and to shed light on the possible roles of mitochondrial electron transport dysfunction in the biogenesis. It should be noted that large amounts of CH4 may be produced by anaerobic fermentation in the mammalian large intestine. In consequence of its physicochemical properties, CH4 traverses the mucosa and freely enters the splanchic microcirculation. It is widely accepted that the bulk of the CH4 production is excreted via the lungs, and breath testing has therefore become a tool for the diagnosis of certain gastrointestinal conditions in humans. Nevertheless, CH4 is distributed evenly across membrane barriers. The pulmonary route is therefore certainly not exclusive, and the production is reflected not only in the exhaled air but also in its passage through body surfaces: a recent study demonstrated the uniform release of CH4 through the skin in healthy individuals (31). It follows that determination of the whole body CH4 output is required for an assessment of the magnitude of the release or clearance. To date, however, no studies have been reported in which the overall CH4 generation was investigated or characterized in vivo. We have therefore designed an experimental setup with which to measure the whole body CH4 output in small animals. We applied photoacoustic spectroscopy with a near-infrared diode laser technique for real-time measurements of CH4 emission in the rat.

In the first part of the study, we set out to determine the in vivo CH4 production profile of the animals under baseline conditions and after the induction of mitochondrial distress by chronic inhibition of mitochondrial cytochrome c oxidase. Changes in leukocyte reactions were chosen as endpoints via which to characterize the proinflammatory potential of the NaN3 protocol. These phenomena coexist in the inflammatory milieu, and various data suggest a multiple connection between them in oxido-reductive stress-induced inflammation and evolving tissue injury.

In the second part of our study, our aim was to modulate the outcome of NaN3-induced chemical hypoxia and additionally to outline a possible mechanism linked to the expected CH4 generation. Here, we took into account the earlier in vivo findings that 1-α-glycerylphosphorylcholine (GPC), a water-soluble, decayed phosphatidylcholine (PC) derivative, proved to be effective against lipid peroxidation and loss of the membrane function in phenomenon can be mimicked by sodium azide (NaN3) administration (17, 41), when selective and stable inhibition of mitochondrial cytochrome c oxidase leads to chemical hypoxia with subsequent energy depletion (6, 25, 38). Although an in vivo biological role for the endogenous generation of CH4 has not been fully explored, our recent data demonstrated that exogenous CH4 confers protection against the development of inflammation following an ischemia-reperfusion insult (7).
SODIUM AZIDE-INDUCED METHANE GENERATION

The instrument was calibrated with various gas mixtures prepared by dilution of 1 vol% of CH₄ in synthetic air (Messer, Budapest, Hungary), and it proved to have a dynamic range of four orders of magnitude; the minimum detectable concentration of the sensor was found to be 0.25 ppm (3σ), with an integration time of 12 s.

For the animal experiments, a specially designed sampling chamber with an internal volume of 2.510 cm³ was used. The chamber could be closed hermetically but was fitted with a device that allowed extraction of a sample of the gas in the chamber for external analysis. Before the animals were placed into the chamber, the CH₄ concentration of the gas in the chamber (room air) was determined and used as baseline in the calculations of the CH₄ emission of the animals. By means of a membrane pump (Rietschle Thomas, Puchheim, Germany) and a mass-flow controller, a sample of the gas from the chamber was drawn through the photoacoustic cell via a tube made of stainless steel. A rat was next placed in the chamber, which was then sealed. It was found that a period of 10 min was sufficient for the level of CH₄ released by the animal (through the skin or exhaled) to be measured reliably and reproducibly in an extraction sample. Accordingly, a sample of the chamber gas was taken for analysis exactly 10 min after the animal had been sealed in the chamber. The rat was then removed from the chamber until the measurements on the subsequent day. The chamber was thoroughly flushed with room air before the next rat was inserted. The CH₄ emission data for each day were taken as the means ± SD for all of the animals. The whole body CH₄ emission of the animal was calculated as the difference in the CH₄ concentration of the sample taken at 10 min and the baseline concentration, referred to the body surface [F (dm²) = 10 × S⁰.⁷⁵ kg].

Intravital video microscopy. The rats were anesthetized with sodium pentobarbital (60 mg/kg ip), and the right jugular vein and carotid artery were cannulated for fluid and drug administration and for the measurement of arterial pressure (a Statham P23Db transducer with a computerized data acquisition system; Experimetria, Budapest, Hungary), respectively. The animals were placed in a supine position on a heating pad to maintain the body temperature between 36 and 37°C, and Ringer’s lactate was infused at a rate of 10 ml·kg⁻¹·h⁻¹ during the experiments, together with small supplementary doses of pentobarbital intravenously when necessary. The trachea was cannulated to facilitate respiration, and after laparotomy the liver was perfused horizontally on an adjustable stage and superfused with 37°C saline. The microcirculation of the liver surface was visualized by means of intravital video microscopy (IVM), using a Zeiss Axioshot Vario 100HD microscope (100-W HBO mercury lamp, Acroplan ×20 water immersion objective). FITC (0.2 ml iv; Sigma) was used for the ex vivo labeling of erythrocytes, and rhodamine-6G (0.2%; 0.1 ml iv; Sigma) was used for the staining of polymorphonuclear leukocytes. The microscopic images were recorded with a charge-coupled device videocamera (AVT HORN-BC 12; Horn Imaging, Aalen, Germany) attached to an S-VHS videorecorder (Pana sonic AG-MD 830; Yokohama, Japan) and a personal computer.

Video analysis. Quantitative assessment of the microcirculatory parameters was performed off-line by frame-to-frame analysis of the videotaped images, using image analysis software (IVM; Pictron, Budapest, Hungary). The red blood cell velocity (RBCV; μm/s) was measured in five separate fields in five sinusoids. The functional capillary density (FCD) was defined as the total length of red blood cell-perfused capillaries per observation area (cm²/cm³). Leukocyte-endothelial cell interactions were analyzed within five central venules of the liver (diameter between 11 and 20 μm per animal). Adherent leukocytes (stickers) were defined in each vessel segment as cells that did not move or detach from the endothelial lining within an observation period of 30 s and are given as the number of cells per millimeter squared of endothelial surface.

In vivo histology. The dynamic structural changes of the liver were investigated by real-time laser scanning confocal endomicroscopy with an excitation wavelength of 488 nm, the emission being detected at 505–585 nm (FIVEL; Optiscan Pty, Notting Hill, Australia). The...
chosen areas were scanned in a raster pattern to construct a transverse optical section (1 scan per image, 1,024 × 512 pixels and 475 × 475 μm per image). The optical slice thickness was 7 μm; the lateral and axial resolution was 0.7 μm. The liver architecture was examined in vivo following topical application of the fluorescent dye acridine orange (Sigma-Aldrich). The objective of the device was placed onto the liver surface, and confocal imaging was performed 5 min after dye administration. Thirty to fifty pictures were stored in each experiment. The thickness of the sinusoids was quantified by image analysis; the qualitative lobular changes were analyzed by using a semiquantitative scoring system. The grading was performed with three criteria: the structural changes of the sinusoids (score 0 = normal; 1 = dye extravasation, but the vessel structure is still recognizable; 2 = destruction, the vessel structure is unrecognizable), edema (score 0 = no edema; 1 = moderate epithelial swelling; 2 = severe edema), and the hepatocyte cell outlines (score 0 = normal, well-defined outlines; 1 = blurred outlines; 2 = lack of normal cellular contours).

Intestinal xanthine oxidoreductase activity. Small intestinal biopsies kept on ice were homogenized in phosphate buffer (pH 7.4) containing 50 mM Tris-HCl (Reanal, Budapest, Hungary), 0.1 mM EDTA, 0.5 mM dithiothreitol, 1 mM PMSF, 10 μg/ml soybean trypsin inhibitor, and 10 μg/ml leupeptin. The homogenate was centrifuged at 4°C for 20 min at 24,000 g, and the supernatant was loaded into centrifugal concentrator tubes. The activity of xanthine oxidoreductase (XOR) was determined in the ultrafiltered supernatant by fluorometric kinetic assay (4).

Intestinal and lung tissue myeloperoxidase activity. The activity of myeloperoxidase (MPO), a marker of polymorphonuclear leukocyte activation, was determined in ileal and lung biopsies. Samples were homogenized with Tris-HCl buffer (0.1 M, pH 7.4) containing 0.1 mM PMSF to block tissue proteases and then centrifuged at 4°C for 20 min at 24,000 g. The enzyme reaction mixture containing 50 mM K2PO4 buffer (pH 6.0), 2 mM 3,3′,5′-tetramethylbenzidine (dissolved in DMSO), and 100 μl of homogenate supernatant was incubated for 5 min at 37°C. The reaction was started with 0.6 mM hydrogen peroxide (H2O2; dissolved in 0.75 ml of K2PO4 buffer) and was stopped after 5 min with 0.2 ml of H2SO4 (2 M), and the H2O2-dependent oxidation of tetramethylbenzidine was detected spectrophotometrically at 450 nm (UV-1601 spectrophotometer; Shimadzu, Kyoto, Japan). MPO levels were calculated via a calibration curve prepared with standard MPO (Sigma-Aldrich). The data were referred to the protein content.

ATP measurements. A sample was taken from the liver, cooled in liquid nitrogen, and stored at −70°C. Afterwards, the sample was weighed, placed into a threefold volume of trichloroacetic acid (6% w/v), homogenized for 1 min, and centrifuged at 5,000 g. After adjustment of the pH to 6.0 with saturated K2CO3 solution, the reaction mixtures were prepared by the addition of 100 μl of ATP assay mix (containing firefly luciferase, luciferin, MgSO4, EDTA, DTT, and BSA in a Tricine buffer; Sigma-Aldrich) to 100 μl of fivefold diluted sample. The ATP determinations were based on the measurement of luciferase chemiluminescence, using a luminometer (LUMAT LB 9507; Berthold Technologies, Bad Wildbad, Germany). ATP levels were calculated with the aid of a standard ATP calibration curve (Sigma-Aldrich). The data were referred to the sample weights.

Statistical analysis. Data analysis was performed with a statistical software package (SigmaStat for Windows; Jandel Scientific, Erkrath, Germany). Friedman repeated-measures ANOVA on ranks was applied within groups. Time-dependent differences from the baseline (0 min) for each group were assessed by Dunn’s method, and differences between groups were analyzed with Kruskal-Wallis one-way ANOVA of variance on ranks, followed by Dunn’s method for pairwise multiple comparisons. In the figures, median values and 75th and 25th percentiles are given. P values < 0.05 were considered significant.

RESULTS

Whole body CH4 release. We performed repeated analyses at 2-day intervals to follow the CH4 profile of each animal (Fig. 1). Chronic NaN3 administration significantly increased the whole body generation of CH4 by day 3 of treatment [median value (M): 2.082 delta parts per million (dppm)/1,000 dm2; 25th percentile (p25): 1.992 dppm/1,000 dm2; and 75th percentile (p75): 2.277 dppm/1,000 dm2], and the higher CH4 output persisted until the end of the experiments (day 8: M: 2.974 dppm/1,000 dm2; p25: 2.630 dppm/1,000 dm2; and p75: 3.362 dppm/1,000 dm2). A statistically significant increase in CH4 release was observed on day 8 in the antibiotic-treated animals subjected to the NaN3 challenge (M: 2.224 dppm/1,000 dm2; p25: 1.528 dppm/1,000 dm2; and p75: 2.346 dppm/1,000 dm2), whereas no elevation was noted in the sham-operated (M:1.337 dppm/1,000 dm2; p25: 1.078 dppm/1,000 dm2; and p75: 1.598 dppm/1,000 dm2) or antibiotic-treated (M:1.598 dppm/1,000 dm2; p25: 0.938 dppm/1,000 dm2; and p75: 1.673 dppm/1,000 dm2) control groups.

More importantly, a significant CH4 level elevation was not demonstrated in the GPC + NaN3-treated group (M: 1.11 dppm/1,000 dm2; p25: 0.83 dppm/1,000 dm2; and p75: 1.437 dppm/1,000 dm2) compared with the matching controls. The elimination of the intestinal bacteria led to a considerable decrease in CH4 emission, but it remained measurable (M: 1.135 dppm/1,000 dm2; p25: 0.848 dppm/1,000 dm2; and p75: 1.312 dppm/1,000 dm2) and by day 8 the level was significantly higher in the animals subjected to the NaN3 challenge than in the control group.

Liver microcirculation. The hepatic microcirculation is well known to be particularly sensitive to inflammatory damage and chemical hypoxia, and the NaN3-induced changes in RBCV and FCD were therefore monitored (Fig. 2). In group 2, the
RBCV in the sinusoids was very low (M: 314.5 \mu m/s; p25: 290 \mu m/s; and p75: 348 \mu m/s), relative to the sham-operated value (M: 812 \mu m/s; p25: 777 \mu m/s; and p75: 839 \mu m/s). After GPC administration, the RBCV increased significantly but did not reach the control level (M: 444 \mu m/s; p25: 408 \mu m/s; and p75: 616.5 \mu m/s). A similar tendency was observed in antibiotic + NaN3-treated group 5. No differences in FCD were found between the groups (data not shown).

Leukocyte-endothelial cell interactions. By day 8, the number of sticking leukocytes in the central venules was markedly enhanced in some of the NaN3-treated animals, but the increase was not significant statistically (P = 0.051) due to the large interindividual differences (data not shown). The results indicated that the extent of leukocyte adhesion did not differ in the GPC + NaN3-treated group from that in the untreated controls (data not shown).

In vivo morphological changes. The structure of the liver was evaluated by means of in vivo imaging, using confocal laser scanning endomicroscopy. The NaN3 treatment itself did not alter the thickness of the sinusoids in the chosen areas (data not shown). The in vivo histology of the rats treated with NaN3 did not reveal any tissue damage, and there were no visible differences in the integrity of the hepatic portal triads between the control and treated groups (Fig. 3).

MPO activities of the ileum and the lung. The MPO produced by the activated leukocytes was chosen as an indicator of the general inflammatory profile of the rat tissues. As reflected in Fig. 4, we observed a statistically significant increase in lung MPO in NaN3-treated group 2 (M: 570 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 544.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 805.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)) and in antibiotic + NaN3-treated group 5 (M: 501.7 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 383.8 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 1,268.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)) compared with the sham-operated control or the GPC-supplemented group (M: 300.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 277.3 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 305.9 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)). In the GPC-gavaged group, the MPO activity was even lower than in the control groups.

Quantification of the MPO activity in the ileum revealed significant elevations in the NaN3-treated animals (M: 1,271.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 1,154.4 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 1,366.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)) and the antibiotic-gavaged NaN3-treated groups (M: 1,274.6 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 782.3 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 1,828.6 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); Fig. 4). GPC supplementation resulted in a significantly lower MPO activity (M: 914.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 783.3 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 944.9 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)) and the data did not differ from those for the sham-operated group (M: 818.3 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 801.7 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 866.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)).

XOR activity in the small intestine. The activation of XOR during hypoxia or ischemia-reperfusion events leads to the production of high amounts of ROS. Thus small intestinal XOR was chosen as a further endpoint via which to characterize the inflammatory potential of NaN3 administration. By day 8 of the experiments, a significantly higher lung XOR activity was noted in animals subjected to the NaN3 challenge (M: 316.2 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 240.9 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 535.4 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)) compared with group 3 (M: 153.9 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p25: 121 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p75: 198.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)) and group 4 (M: 216.9 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); and p25: 187.1 pmol·min \(^{-1} \cdot \text{mg}^{-1} \); p75: 243.5 pmol·min \(^{-1} \cdot \text{mg}^{-1} \)). The increase was statistically not significant in the antibiotic + NaN3-treated group 5 compared with the matching control.

Liver ATP level. To establish whether NaN3 influences the mitochondrial function, we quantified liver ATP production on day 8 of the chronic challenge. The results indicated a significant ATP depletion in group 2 (M: 0.122 nmol·ml \(^{-1} \cdot \text{mg}^{-1} \); p25: 0.089 nmol·ml \(^{-1} \cdot \text{mg}^{-1} \); p75: 0.189 nmol·ml \(^{-1} \cdot \text{mg}^{-1} \)) and in the sham-operated group (Fig. 6). The ATP level in the liver of the GPC-gavaged group was higher, but the increase was not significant statistically (M: 0.199 nmol·ml \(^{-1} \cdot \text{mg}^{-1} \); p25: 0.182 nmol·ml \(^{-1} \cdot \text{mg}^{-1} \); and p75: 0.222 nmol·ml \(^{-1} \cdot \text{mg}^{-1} \)) compared with either the NaN3-treated or the antibiotic-gavaged + NaN3-treated group. The GPC-treated animals produced similar amounts of ATP as observed in the control groups.
DISCUSSION

CH$_4$, the most reduced form of carbon, plays an important role in both tropospheric and stratospheric chemistry (20), but the significance of endogenous CH$_4$ production in cellular physiology is still not known for certainty. Mammalian methanogenesis is closely associated with the activity of intestinal anaerobic bacteria; however, previous studies have demonstrated the generation of nonbacterial CH$_4$ in aerobic living systems as well (16, 17, 23, 24). In 2003 we reported on hypoxic mitochondrial CH$_4$ generation (16), and in 2006 Keppler et al. (24) described direct CH$_4$ emission from plants under aerobic conditions. This was followed by many studies that either supported or disagreed with the initial findings (10, 13, 28, 29), but several stable isotope studies have now confirmed the possibility of plant-derived nonbacterial CH$_4$ formation (9, 41).

Here we assumed that CH$_4$ excretion in the breath reflects intestinal bacterial fermentation plus an unknown and variable amount of nonbacterial generation induced from target cells. Secondly, we hypothesized that if nonbacterial CH$_4$ is added to the bacterial production, this addition could occur at such a rate that it is impossible to detect it by the conventional techniques utilized to look for it to date. During this study, we performed an in-depth range of biochemical investigations with matching in vivo analysis techniques to determine the magnitude of the whole body CH$_4$ emission of NaN$_3$-treated rats and compared the profile with that found in the untreated animals. The CH$_4$ exhaled from the airways together with the amounts discharged from the skin, and body orifices was quantified by means of whole body photoacoustic spectroscopy. Through determination of the amounts of CH$_4$ released from the animals at the different times, our study demonstrated that chronic NaN$_3$...
administration was accompanied by an increasing emanation of endogenous CH₄ throughout the entire duration of the experiments.

The main effect of NaN₃ is the direct inhibition of the activity of the mitochondrial electron transport chain through irreversible binding to the heme cofactor of cytochrome c oxidase (6); thus it can be considered a specific tool with which to study mitochondrial oxido-reductive stress. It is well established that NaN₃ administration can lead to the production of mitochondrial ROS in different experimental setups (22, 34, 40). In our study, the NaN₃-induced global mitochondrial dysfunction was evidenced by hepatic ATP depletion, and a systemic inflammatory reaction. Direct in vivo evidence was also obtained also for the deranged liver microcirculation, while the higher XOR and MPO activities indirectly demonstrated the impact of cytochrome c oxidase inhibition on ROS generation in several tissues. It is also important to mention that significant CH₄ formation was detected, irrespective of the concomitant antibiotic treatment targeting the potentially CH₄-producer gastrointestinal bacterial flora. Thus the overall evidence from these findings suggests that the CH₄-generating capacity of NaN₃ administration is independent of the methanogenic archae but may be associated with the NaN₃-induced generation of potentially damaging ROS.

The mechanism and significance of the nonbacterial CH₄-forming reaction are still not fully elucidated, mainly because many possible sources and various unknown reaction pathways can be envisaged. The initial in vitro studies led to the proposal that electrophilic methyl groups (EMG) bound to positively charged nitrogen moieties (as in choline molecules) may potentially act as electron acceptors, and that these reactions may entail the generation of CH₄ (15, 16). A continuous lack of the electron acceptor O₂ will maintain an elevated mitochondrial NADH-to-NAD⁺ ratio, causing reductive stress and formation of a nucleophilic hydride ion, which may be transferred to the EMG (15). Thus priming during hypoxia occurs as a progressive process involving depressed electron transport in the setting of PC breakdown, the loss of cytochrome c and antioxidants and the triggering of CH₄ release during reoxygenation or reperfusion. Therefore, it is possible that the formation and constant building-up of ROS in the mitochondria are part of a reaction, which furnishes CH₄.

The mitochondria are either targets or sources of oxido-reductive stress. In the second part of our protocol, therefore, we set out to influence the potentially detrimental process that leads to the collapse of the energy-producing cellular system. We demonstrated the ability of GPC to effectively silence several inflammatory consequences linked to a reaction that might involve cellular or mitochondrial ROS generation. GPC is a centrally acting cholinergic precursor that increases the tolerance to ischemic tissue damage (32). Clinically, it is effective in cerebrovascular and neurodegenerative diseases (21, 33), including ischemic stroke (2). More importantly, GPC can act as choline source in various tissues (1, 3, 27). When this water-soluble, deacylated PC analog was administered in chemical hypoxia, the microcirculatory dysfunction, the increase in the activity of the ROS-producer XOR, and the accumulation of leukocytes were all moderated. Moreover, the extent of CH₄ generation in NaN₃-treated animals was reduced concomitantly. It should be added that exogenous PC also exerted an anti-inflammatory influence in the gastrointestinal tract and significantly decreased the exhaled methane concentration in a canine and rat model of intestinal ischemia-reperfusion (18, 26). These data clearly suggest that CH₄ release is an indicator of hypoxia-induced pathologies and also imply that GPC may be effective against such injuries.

The mechanistic role of hypoxia-induced CH₄ generation remains to be established, but a possible explanation could be an endogenous need for the sparing or regeneration of membranes. Complex IV inhibition causes ROS production and serious membrane loss due to ROS-induced lipid peroxidation. Peroxidation is an immediate chain reaction; in a short time it causes a fundamental breakdown of biomembranes, leading to decompartmentalization, loss of integrity, and cell death. As a consequence, membrane sparing and recovery are particularly important tasks in oxido-reductive environments. To avoid the potentially fatal outcome of an increased oxido-reductive potential, molecular participants of a living system should be quickly brought into use to save or regenerate membranes, which are responsible not only for separation, but also for the maintenance of a steady state via channels, pores, and membrane proteins. Theoretically, all of the molecules which are potential components of phospholipid bilayers might be reutilized. During such processes, compounds rich in ethyl and methyl groups can be reduced by electron acceptance. This yields molecules used to seal or build up membranes, together with fully reduced CH₄ (15). Thus we propose that CH₄ is the end product of a protective mechanism linked to membrane defense or regeneration during ROS-induced damage. The outcome of GPC treatment reinforces this conception, since the level of CH₄ formation was lower and the inflammatory reaction secondary to chemical hypoxia was diminished in the GPC-treated animals.

GPC is the most bioavailable source of choline (3), which can be directly or indirectly involved in the reconstruction of injured phospholipid bilayers. Accordingly, in the presence of higher intracellular GPC concentrations, the degradation of endogenous, membrane-forming compounds that could eventually have resulted in the emission of CH₄ was reduced. However, it is not clear whether GPC selectively influences CH₄ metabolism because it can modulate other processes (e.g., blood flow-dependent or other biochemical pathways) involved in ischemia-reperfusion-mediated injury. Indeed, Cao et al. (11) have reported that the PC metabolism in the rat heart is regulated by vitamin E, and additionally, it was shown that vitamin E is less effective against oxido-reductive stress if no added PC is present (37). This suggests that GPC can also cooperate with the vitamin E-mediated antioxidant protection by stabilizing membranes.

In conclusion, we detected whole body CH₄ generation in real-time with photoacoustic spectroscopy in rodents. With this technique, the daily CH₄ production profile can be determined and stress-caused changes or treatment effects can be evaluated accurately and reproducibly. This setup revealed that mitochondrial cytochrome c oxidase inhibition with chronic NaN₃ administration resulted in a significant elevation in the CH₄ output, together with activation of an inflammatory response. Accordingly, we propose that CH₄ emission in mammals may be connected with hypoxic events leading to, or associated with a mitochondrial dysfunction. In the GPC-treated animals, the production of CH₄ was kept at the level in the sham-
operated group, which points to a role of CH₄ as an alarm signal for the development of mitochondrial responses under hypoxic conditions and a possible indication for GPC administration to influence such events.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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