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## Paracetamol (Acetaminophen) Decreases Hydrogen Sulfide Tissue Concentration in Brain but Increases It in the Heart, Liver and Kidney in Mice\*

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The biological action of N-acetyl-p-aminophenol - paracetamol (acetaminophen) has been demonstrated to involve different mechanisms and is still not clear. Hydrogen sulfide (H<sub>2</sub>S) has been shown to play an important role in many physiological and pathological processes including nociception. The interaction between acetaminophen and endogenous H<sub>2</sub>S is unknown. Twenty four female CBA strain mice were administered intraperitoneal injections of N-acetyl-p-aminophenol solution: paracetamol in doses of 30 mg/kg b.w. per day (group D1, n = 8) or 100 mg/kg b.w. per day (group D2, n = 8). The control group (n = 8) received physiological saline in portions of the same volume – 0.2 ml. The measurements of tissue H<sub>2</sub>S concentration were performed with the Siegel spectrophotometric modified method. In the brain, the H<sub>2</sub>S tissue level decreased, but more significantly in the lower drug dose group. Conversely, there was a significant rise in the H<sub>2</sub>S tissue concentration in D1 and D2 groups in heart and kidney with the increase more pronounced in the group with the lower paracetamol dose. In the liver only the higher acetaminophen dose elicited a change in H<sub>2</sub>S concentration, increasing after administration of acetaminophen at 100 mg/kg. Our study demonstrates that paracetamol induces H<sub>2</sub>S tissue concentration changes in different mouse organs.

Key words: Paracetamol, acetaminophen, hydrogen sulfide, pain, nociception.

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N-acetyl-p-aminophenol – paracetamol (acetaminophen) is an example of a paradox in medicine. Despite lacking basic knowledge of the mechanisms of action of paracetamol, its antipyretic and analgetic properties have been successfully used, including pediatrics and over-the-counter sale, for over 50 years (BERTOLINI *et al.* 2006). Also paradoxically, studies of the recent decade have changed the perception of hydrogen sulfide (H<sub>2</sub>S) from a rotten egg odorous poisonous gas to a ‘gas-transmitter’ and an important co-regulator of nu-

merous endogenous processes in mammals (ŁOWICKA & BELTOWSKI 2007). Since paracetamol affects some processes that H<sub>2</sub>S is recognized to co-modulate, like nociception, the question arises if the biology of acetaminophen involves H<sub>2</sub>S (LEE *et al.* 2008). The influence of paracetamol on H<sub>2</sub>S generation and the role of the gas-transmitter in the biological effects of the drug are unknown. The aim of this study was to assess the impact of acetaminophen on the tissue H<sub>2</sub>S concentration in mouse brain, heart, liver and kidney.

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## Material and Methods

### Animals

Twenty four CBA strain female mice (3-4 month 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 a temperature of 22-24°C with a light/dark cycle of 12 h.

### Study protocol

The study has been performed in accordance with the guidelines for the care and use of laboratory animals accepted by the Bioethical Committee of the Jagiellonian University Medical College.

The study design comprised intraperitoneal administration of N-acetyl-p-aminophenol of 30 mg/kg body weight per day (0.6 mg/d – group D1, n = 8) or 100 mg/kg body weight per day (2.0 mg/d – group D2, n = 8) for 5 consecutive days at the same time of the day (10:30 am). N-acetyl-p-aminophenol (Paracetamol, Biofarm, Poland) was dissolved in physiological saline (each injection contained 0.2 ml of the solution). The control group (n = 8) 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 paracetamol well and remained in good condition till the end of the experiment. Measurements of H<sub>2</sub>S concentration were performed by the use of the modified method of Siegel (SIEGEL 1965; SOMOGYI *et al.* 2008).

### Tissue sample preparation

The animals were killed by cervical dislocation 2 hours after the last drug or physiological saline injection. Their brains, hearts, livers and kidneys were quickly removed and homogenized with 0.01 mol/l sodium hydroxide (NaOH): brain tissue in proportion of 1 to 3, liver of 1 to 5, heart and kidney of 1 to 10 and frozen. Then 50% trichloroacetic acid

(TCA) was added (0.5 ml to 2 g of brain or liver samples in tight capsules of 3 ml and 0.25 ml to 1 g of heart or kidney samples in tight capsules of 2 ml), the suspension was shaken and centrifuged. Subsequently, 1.5 ml of 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 mol/l N,N-dimethyl-p-phenyl-diamine sulfate in 7.2 mol/l hydrochloric acid (HCl), then 0.15 ml or 0.075 ml of 0.03 mol/l iron (III) chloride (FeCl<sub>3</sub>) in 1.2 mol/l HCl portions were added, respectively. After 20 minutes in darkness the content was shaken for 1 minute with 1 ml of chloroform.

### H<sub>2</sub>S tissue concentration measurement

Absorbance was measured at 650 nm with the Varian Cary 100 spectrophotometer. A standard curve was prepared with an iodometrically determined 0.0001 mol/l sodium sulfide (Na<sub>2</sub>S) solution. For each group of animals four concurrent analyses of every analyzed tissue type were performed.

### Statistical analysis

Statistical analysis was performed within the R Environment by the Student's *t*-test. Statistical significance was considered when P < 0.05.

## Results and Discussion

There was a significant rise in the H<sub>2</sub>S tissue concentration in D1 and D2 groups in the heart and kidney, the increase was more pronounced in the group with the lower paracetamol dose as compared to the group of drug higher dose. Conversely, in the brain the H<sub>2</sub>S tissue level decreased but more in the lower acetaminophen dose group. In the liver only a higher paracetamol dose evoked a H<sub>2</sub>S concentration change, i.e. its level increased after 100 mg/kg of acetaminophen administration. The results are presented in Table 1.

Table 1

Hydrogen sulfide (H<sub>2</sub>S) tissue concentrations in mouse brain, heart, liver and kidney following administration of 30 mg/kg b.w./d or 100 mg/kg b.w./d of paracetamol (groups D1 and D2 respectively)

H <sub>2</sub> S tissue concentration (μg/g)	Control group (n = 8)	D1 (n = 8)	P (control vs D1)	D2 (n = 8)	P (control vs D2)
Brain	1.47 ± 0.02	0.80 ± 0.02	<0.01	1.05 ± 0.02	<0.01
Heart	6.89 ± 0.14	8.12 ± 0.16	<0.01	7.35 ± 0.10	<0.01
Liver	3.92 ± 0.06	3.95 ± 0.07	0.54	4.65 ± 0.09	<0.01
Kidney	7.13 ± 0.07	9.60 ± 0.14	<0.01	8.36 ± 0.14	<0.01

A debate about paracetamol's primary site of action has been ongoing in the literature for years. Much investigation has focused on acetaminophen's inhibition of prostaglandin (PG) synthesis, because its analgesic and antipyretic properties resemble the effects of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Synthesis of prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) is a crucial stage in arachidonic acid metabolism and is catalyzed by two major forms of PGH<sub>2</sub> synthetase (PGHS) – constitutive PGHS-1 and inducible PGHS-2. PGHS contains two sites: a cyclooxygenase (COX) site and a peroxidase (POX) site. Paracetamol acts as a reducing cosubstrate on the POX site and lessens the availability of the ferryl protoporphyrin IX radical cation, essential for conversion of tyrosine-385 to its radical form, which is necessary for the transformation of arachidonic acid to prostaglandin G<sub>2</sub> (PGG<sub>2</sub>) on the COX site. What is noteworthy, this effect can be reduced in the presence of hydroperoxide-generating lipoxygenase enzymes within the cell (peroxide tone) or by swamping the POX site with substrate such as PGG<sub>2</sub> (ANDERSON 2008). Some hope was also associated with the discovery of a variant of PGHS-1 (named PGHS-1b or COX-3), sensitive to inhibition with paracetamol and active in the cerebral cortex of canines, but subsequent research found it improbable to play a role in PG-mediated fever and pain in humans and mice (CHANDRASEKHARAN *et al.* 2002; KIS *et al.* 2005).

As paracetamol seems to not have major effects peripherally, its action appears to be mostly central. Peroxide tone and swamping might explain the lack of peripheral analgesic, platelet and anti-inflammatory effects by acetaminophen but alternative PGHS inhibition mechanisms have been also proposed (BERTOLINI *et al.* 2006). Inhibition of the L-arginine – nitric oxide (NO) pathway mediated through substance P or N-methyl-D-aspartic acid (NMDA), reinforcement of descending inhibitory serotonergic pain pathways and active paracetamol metabolites (p-aminophenol conjugated with arachidonic acid by fatty acid amide hydrolase forming AM404) exerting effect through cannabinoid receptors are postulated (ANDERSON 2008; BJORKMAN *et al.* 1994; BUJALSKA 2004; HOGESTATT *et al.* 2005; OTTANI *et al.* 2006; PICKERING *et al.* 2008).

Hydrogen sulfide is endogenously formed from L-cysteine in several enzymatic reactions, catalyzed by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST), and in non-enzymatic pathways in many tissues, especially in the nervous, cardiovascular, digestive and excretory systems. H<sub>2</sub>S participates in the regulation of various physiological and pathophysiological processes including neurotransmission, immune and inflam-

matory processes, and perception (FIORUCCI *et al.* 2006; ŁOWICKA & BELTOWSKI 2007). H<sub>2</sub>S is involved in multiple nociception signaling pathways with increasing cAMP levels in neuronal and glial cell lines, activation of T-type calcium channel and ATP-sensitive potassium channels (K<sub>ATP</sub>), sensitization of the NMDA receptor complex and activation of TRPA1 (transient receptor potential cation channel, subfamily A, member 1) ion channels (CUNHA *et al.* 2008; DISTRUTTI *et al.* 2006; MATSUNAMI *et al.* 2009; SMITH 2009; STRENG *et al.* 2008; TODOROVIC & JEVTOVIC-TODOROVIC 2006). Moreover, H<sub>2</sub>S interacts with NO and carbon monoxide (CO) in a complex manner including affecting each other's synthesis and biological responses within target tissues and organs depending on specific environmental circumstances (LI *et al.* 2009). H<sub>2</sub>S has been shown to display various effects which promote neutrophil migration, upregulation of COX-2 and production of inflammatory mediators with prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) which appears to act on nociceptor membranes (CUNHA *et al.* 1992; HU *et al.* 2008; SMITH 2009). On the other hand, it has been suggested that H<sub>2</sub>S could be produced in the inflammatory site by migrating leukocytes (CUNHA *et al.* 2008).

As we have demonstrated, paracetamol elicits changes in H<sub>2</sub>S tissue concentrations reflecting alterations in the transmitter generation. Since H<sub>2</sub>S has been recognized to be involved in a wide variety of processes of nociception with its predominant role of activation of T-type calcium channels leading to facilitation of pronociceptive actions, the observation that acetaminophen decreases the H<sub>2</sub>S level is quite an enticing finding (ANDERSON 2008). The role of H<sub>2</sub>S mediation of paracetamol's central analgetic effects is definitely a field for future research, especially when considering common features in the biological action of acetaminophen and H<sub>2</sub>S such as involvement of the NMDA receptor, transient receptor potential vanilloid (TRPV) ion channels system and NO. Secondly, H<sub>2</sub>S tissue concentration changes in heart, liver and kidneys indicate that paracetamol exerts peripheral effects in different organs with possible involvement of PGHS. Interaction between prostaglandins and H<sub>2</sub>S seems to be complex, including each other's generation and effects. Moreover, it appears that effects of H<sub>2</sub>S vary depending on the environment and circumstances under which it co-acts with other messengers in the specific setting of different organs.

Paracetamol is another drug shown to affect H<sub>2</sub>S production next to NSAIDs and angiotensin-converting enzyme inhibitor (ACEI) ramipril, encouraging further research of the messenger role in physiology and pathology of different systems

(FIORUCCI *et al.* 2007; SREBRO *et al.* 2006; WILIŃSKI *et al.* 2010; WILIŃSKI *et al.* 2008).

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