

## HYDROGEN SULFIDE (H<sub>2</sub>S) INHIBITS AGGREGATION OF HUMAN PLATELETS

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Hydrogen sulphide (H<sub>2</sub>S) is a newly recognized endogenous gas produced in the body from cysteine mainly by two enzymatic pathways, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (1). H<sub>2</sub>S, similarly to nitric oxide (NO), exerts a wide variety of biological effects at both central and peripheral levels including the vascular system (1). Here, we investigated whether H<sub>2</sub>S affects aggregation of human platelets, using sodium hydrogen sulfide (NaHS) as H<sub>2</sub>S-donor. NaHS inhibited platelet aggregation induced by ADP, 2 μM (IC<sub>50s</sub> 698 μM), collagen, 2 μg/ml (IC<sub>50s</sub> 1.69 mM), epinephrine, 7 μM (IC<sub>50s</sub> 863 μM), arachidonic acid, 145 μg/ml (IC<sub>50s</sub> 859 μM), thrombin, 0.5 U/ml (IC<sub>50s</sub> 759 μM), and the thromboxane mimetic, U46619 (IC<sub>50s</sub> 5.62 mM) (IC<sub>50s</sub>: concentration producing 50% inhibition of full aggregation). IC<sub>50</sub> values of NaHS against the different aggregating stimuli used, exceed the physiological concentrations of H<sub>2</sub>S detected in human plasma (10-100 μM) (2). However, significant inhibition of platelet aggregation induced by moderate ADP concentration (0.8 μM), was obtained with a concentrations of NaHS as low as 30 μM. Preincubation (30 min) with NO-synthase inhibitor, L-NAME (100 μM), adenylyl-cyclase inhibitor SQ 22,536 (100 μM), guanylyl-cyclase inhibitor ODQ (100 μM), glibenclamide (100 μM) and the unselective K<sup>+</sup>-channel blocker, TEA (20 mM), did not affect the action of NaHS. Further, we tested the possibility that the inhibitory effect of NaHS on platelet aggregation was due to a toxic effect of H<sub>2</sub>S. In presence of 10 mM NaHS, aggregation induced by collagen, 2 μg/ml and U46619, 0.5 μM was abated, but it was completely restored if the stimulus concentration was slightly increased (5 μg/ml, 1 μM respectively). Moreover, treatment of platelets with 10 mM of NaHS (10 min) did not show toxic effects determined by a trypan blue dye exclusion test. The mechanism by which NaHS/H<sub>2</sub>S inhibits platelet aggregation remains undetermined. It could be proposed that physiological H<sub>2</sub>S concentrations in plasma may be sufficient to maintain platelets in a condition of refractoriness toward aggregation induced by mild to moderate stimuli. A corollary to this hypothesis is that high levels of H<sub>2</sub>S may favour platelet disaggregation and *vice versa*. In this respect, it is intriguing to note that patients with Down's Syndrome, who have (~50%) increased H<sub>2</sub>S production and low homocysteine level because of CBS hyperactivity (3), show a low incidence of thromboembolic fatal events (4). Further studies are needed to confirm the role of endogenous H<sub>2</sub>S in platelets homeostasis and to assess whether low H<sub>2</sub>S plasma level may represent a risk factor for cardiovascular diseases.

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