



## EFFECTS OF THIMEROSAL, AN ORGANIC SULFHYDRYL MODIFYING AGENT, ON SEROTONIN TRANSPORT ACTIVITY INTO RABBIT BLOOD PLATELETS

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**Abstract**—The effects of the sulfhydryl group inhibitor thimerosal on serotonin (5-HT) transport activity into rabbit blood platelets were investigated, along with its effects on the intracellular concentration of  $\text{Ca}^{2+}$  ( $[\text{Ca}^{2+}]_i$ ).  $^3\text{H}$ -5-HT transport activity into rabbit blood platelets was inhibited by treatment with  $10^{-5}$  M thimerosal for 30 min, which did not cause 5-HT release from platelets. The thimerosal-induced inhibition of 5-HT transport was antagonized by dithiothreitol. It was suggested that the thimerosal acts as a sulfhydryl inhibitor and inhibits 5-HT transport activity independently of the 5-HT release reaction in our experiment using rabbit blood platelets. As aspirin did not affect thimerosal-induced 5-HT transport inhibition, it was suggested that the thromboxane  $\text{A}_2$ -generating system does not operate in the effect of thimerosal on 5-HT transport into blood platelets. Furthermore, thimerosal induced a transient elevation of  $[\text{Ca}^{2+}]_i$ , which was followed by a sustained increase. In the absence of extracellular  $\text{Ca}^{2+}$ , thimerosal caused only a transient increase in  $[\text{Ca}^{2+}]_i$ . It was suggested that the elevation of  $[\text{Ca}^{2+}]_i$  consisted of two phases, e.g. a transient phase induced by  $\text{Ca}^{2+}$  mobilization from the intracellular store sites and a sustained phase which might be explained by  $\text{Ca}^{2+}$  influx from the extracellular environment. In conclusion, thimerosal inhibited 5-HT transport into blood platelets at a concentration which did not induce 5-HT release, and intracellular  $\text{Ca}^{2+}$  mobilization might mediate the inhibitory effect of thimerosal on 5-HT transport. Copyright © 1996 Elsevier Science Ltd.

The organic mercury compound thimerosal is known to modify physiological responses by binding to sulfhydryl and disulfide groups in various cellular systems. In rat hepatocytes, for example, thimerosal promoted the release of  $\text{Ca}^{2+}$  from inositol trisphosphate-sensitive  $\text{Ca}^{2+}$  stores (Missiaen *et al.*, 1991). Thimerosal was shown to inhibit  $\text{Ca}^{2+}$  uptake in skeletal muscle sarcoplasmic reticulum and rat cerebellar microsomes by inhibiting  $\text{Ca}^{2+}$ -ATPase (Sayers *et al.*, 1993). In addition, it was reported that thimerosal may be an alternative agent for studying  $\text{Ca}^{2+}$ -induced- $\text{Ca}^{2+}$ -release (CICR) in caffeine-insensitive cells such as unfertilized hamster eggs (Swann, 1991) and blood platelets (Adunyah, 1986). Hecker *et al.* (1989) reported that thimerosal caused mobilization of  $\text{Ca}^{2+}$  from its intracellular stores, and then induced aggregation and 5-HT release in human blood platelet preparations. Thus, thimerosal has been proposed to be a useful agent to analyze intracellular  $\text{Ca}^{2+}$  move-

ment and its physiological significance in various cellular systems including blood platelets.

5-hydroxytryptamine (5-HT) functions as a neurotransmitter in the mammalian nervous system. 5-HT is known to play an important role in a multitude of cognitive and behavioral (dys)functions including motor control, feeding, anxiety, depression and sexual activity. Regulation of 5-HT transport has been the major focus of antidepressant research, with many specific 5-HT uptake inhibitors being effective antidepressants. Since platelets have a very rapid active transport system for 5-HT which has been shown to have the same pharmacological characteristics as serotonergic nerve endings (Pletscher, 1968), platelets are proposed to be a potential model for 5-HT neurons (Pletscher, 1988). It has been reported that the human platelet 5-HT uptake site is identical to the human brain 5-HT transporter, and that both proteins are encoded by the same single-copy gene which has been assigned to the human chromosome 17 (Lesch *et al.*, 1993). In view of data suggesting abnormal platelet

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