Short Communications

Effect of 5-Hydroxytryptamine on the Potassium Ion Exchange of Human Platelets Enriched in Sialic Acid

By F. MICHAL and G. V. R. BORN

*Department of Pharmacology, Royal College of Surgeons of England,
London WC2A 3PN, U.K.*

and L. MESTER and L. SZABADOS

*Institut de Chimie des Substances Naturelles, Centre National de
la Recherche Scientifique, Gif-sur-Yvette-91, France*

(Received 21 July 1972)

When human platelets are enriched in sialic acid (Mester, 1971) their aggregation by ADP is diminished, whereas aggregation by 5-hydroxytryptamine is accelerated (Mester *et al.*, 1972). This is evidence in favour of the idea that sialic acid is a component of the 5-hydroxytryptamine receptor complex on platelets.

A sensitive criterion of cell membrane function is the rate of active transport of K^+ . Platelets exchange their intracellular K^+ at a rate of about $18 \mu\text{mol/s}$ per 10^{11} platelets, and this rate is increased by up to 24% during the uptake of 5-hydroxytryptamine by the platelets (Born, 1967). This suggested that the reaction between 5-hydroxytryptamine and the platelet membrane in some way accelerates the movement of K^+ into the platelets. In view of the evidence that incorporated sialic acid increases the number of receptor sites for 5-hydroxytryptamine, and to find out whether the incorporation of sialic acid influences K^+ transport into platelets, we have investigated the effect of incorporating sialic acid on

the uptake of radioactive K^+ by platelets in the absence and in the presence of 5-hydroxytryptamine.

Citrated human platelet-rich plasma was prepared by centrifugation (see, e.g., Born, 1967). To each 1 ml of platelet-rich plasma was added 0.1 ml of buffer (pH 7.4) prepared by mixing 9 vol. of 0.25 M-sucrose with 1 vol. of 1% piperazine- N,N' -bis-(2-ethanesulphonic acid) (PIPES), together with $40 \mu\text{l}$ of 10 mM-CMP- N -[^{14}C]acetylneuraminic acid (approx. 3.3×10^6 c.p.m./ μmol) synthesized by the method of Comb & Roseman (1960) and Kean & Roseman (1966) and $9 \mu\text{l}$ of homogenized rat liver (1 g wet wt. of rat liver/3.27 ml of aq. 0.25 M-sucrose soln.) as source of sialyltransferase.

Samples (1 ml) of these mixtures were incubated at 37°C for 30 min (in one experiment also for 10 min) with $10 \mu\text{l}$ of $^{42}\text{K}_2\text{CO}_3$ solution containing $60.5 \mu\text{mol}$ of $^{42}\text{K}^+$ /ml (approx. 3000 c.p.m./ μg of K^+) obtained from the Atomic Energy Research Establishment, Harwell, Berks., U.K. Half of such samples contained 5-hydroxytryptamine (May and Baker Ltd.,

Table 1. *Effect of the incorporation of sialic acid on the influx of $^{42}\text{K}^+$ into platelets in the presence of 5-hydroxytryptamine*

K^+ influx is expressed as percentage increase (+) or decrease (-). Values are means of measurements carried out with duplicate samples. Platelet concentrations were: Expt. 1, 4.27×10^8 /ml; Expt. 2, 3.92×10^8 /ml; Expt. 3, 3.89×10^8 /ml.

Expt. no.	Incubation time (min)	K^+ influx (% increase or decrease)			Increase in sialic acid (%)
		CMP- N -[^{14}C]acetylneuraminic acid only (a)	Buffer control + 5-hydroxytryptamine (b)	CMP- N -[^{14}C]acetylneuraminic acid + 5-hydroxytryptamine (c)	
1	10	-4	+11	+25	+19
	30	-7	+1	+32	+31
2	30	+2	+9	+16	+16
3	30	-3	+9	+24	+23

Dagenham, Essex, U.K.) at a final concentration of $10\mu\text{M}$. In one-quarter of the samples 5-hydroxytryptamine was replaced by 0.9% NaCl and in another one-quarter of the samples CMP-*N*-acetylneuraminic acid solution and the rat liver homogenate were replaced by equal volumes of 0.25M-sucrose.

At the end of the incubation period the platelets were separated by centrifugation and extracted, and the amount of $^{42}\text{K}^+$ exchanged was determined by liquid-scintillation counting (Born, 1967). The sialic acid incorporated was determined by radioactivity measurement after high-voltage electrophoresis (Mester, 1971).

The results are shown in Table 1 (columns *a-d*). In three experiments the sialic acid content of the platelets, determined as bound [^{14}C]sialic acid, increased by 19–31% when compared with average normal values (Mester *et al.*, 1971). This increase did not cause an increase, indeed if anything there was a slight decrease, in the uptake of radioactive K^+ (column *a*). In platelets without extra sialic acid the presence of 5-hydroxytryptamine always increased K^+ uptake

(column *b*), confirming the previous observation (Born, 1967). With extra sialic acid this increase was further augmented at least twofold (column *c*). The percentage increases in sialic acid and K^+ uptake were similar (column *d*).

These results support the conclusion that the 5-hydroxytryptamine receptor of human platelets contains sialic acid, and they suggest further that the reaction between 5-hydroxytryptamine and this receptor accelerates the movement of K^+ into the platelets.

Born, G. V. R. (1967) *J. Physiol. (London)* **190**, 273

Comb, D. G. & Roseman, S. J. (1960) *J. Biol. Chem.* **235**, 2529

Kean, E. L. & Roseman, S. J. (1966) *J. Biol. Chem.* **241**, 5643

Mester, L. (1971) in *Platelet Aggregation* (Caen, J., ed.), p. 131, Masson et Cie., Paris

Mester, L., Szabados, L., Guinebault, P. R., Caen, J. & Levy-Toledano, S. (1971) *C.R. Acad. Sci. Ser. D* **273**, 2157

Mester, L., Szabados, L., Born, G. V. R. & Michal, F. (1972) *Nature (London) New Biol.* **236**, 213