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Short Communications

Accelerated Uptake of 5-Hydroxytryptamine by Human Blood Platelets Enriched in a Sialic Acid

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The enzymically catalysed incorporation of *N*-acetylneuraminic acid into human platelets, whether suspended in their own citrated plasma or in buffered saline containing 0.17mM-sucrose, accelerated the uptake of 5-hydroxytryptamine. This acceleration decreased with time. The observations may be explained by assuming that *N*-acetylneuraminic acid is a component of a transport receptor for 5-hydroxytryptamine.

Incorporation of *N*-acetylneuraminic acid labelled with ^{14}C into the platelet membrane increases the velocity of platelet aggregation by 5-hydroxytryptamine but not by ADP (Mester *et al.*, 1972) and also increases the influx of K^+ into the platelets in the presence of 5-hydroxytryptamine (Michal *et al.*, 1972). Both effects suggest that this sialic acid is a component of a primary receptor for 5-hydroxytryptamine on the platelet membrane.

Recent evidence (Born *et al.*, 1972) suggested that the receptor which mediates platelet aggregation by 5-hydroxytryptamine is different from that which mediates uptake of the amine by platelets. We have therefore investigated the effect of the incorporation of *N*-acetylneuraminic acid on the uptake of radioactive 5-hydroxytryptamine by platelets.

Platelet-rich plasma was prepared from human citrated blood (see, e.g., Born & Hume, 1967). Samples (6ml) of platelet-rich plasma were mixed with 0.6ml of a buffer at pH7.4 consisting of 9 vol. of 0.25M-sucrose and 1 vol. of Pipes [1% piperazine-*NN'*-bis-(2-ethanesulphonic acid)], 60μl of sialyltransferase preparation and 240μl of 10mM-CMP-*N*-acetyl[^{14}C]neuraminic acid (approx. 3.4×10^6 c.p.m./μmol) synthesized by the method of Kean & Roseman (1966). Sialyltransferase was prepared from rat liver and purified by the method of Schachter *et al.* (1970). Control samples contained only the buffer. It has been previously shown that *N*-acetylneuraminic acid is not incorporated into platelets in the absence of sialyltransferase (Mester, 1971).

After incubation at 37°C for 10min the samples were divided into three parts. These smaller samples of 2ml each were then further incubated for 5, 20 or 30min with 5-hydroxy[^{3}H]tryptamine creatinine sulphate (The Radiochemical Centre, Amersham, Bucks., U.K.) at a final concentration of 5μM. During incubation the pH of the samples was adjusted to 7.4 with 1mM-$NaHCO_3$ solution. After incubation the platelets were separated by centrifugation or by high-voltage electrophoresis (4000V, 45min) with Shell 505 aliphatic solvent and 1% sodium tetraborate buffer, pH6.3, on Whatman 3MM paper (Mester, 1971). Incorporation of sialic acid and uptake of 5-hydroxytryptamine were measured by scintillation counting of ^{14}C and ^3H in the Packard Tri-Carb spectrometer, by using the double-isotope technique.

The results are shown in Table 1. The incorporation of *N*-acetylneuraminic acid increased with time, apparently to a maximum of about 29nmol/10^9 platelets within 40min. Platelets so enriched with *N*-acetylneuraminic acid took up 5-hydroxytryptamine more rapidly than did control platelets. Thus the enriched platelets took up 76% more during the first 5min of incubation, 58% more after 20min and 29% more after 30min than did the control platelets.

Platelets separated from plasma and washed twice in buffer (Cronberg & Caen, 1971) were resuspended in a solution of 0.17mM-sucrose in 0.138M-NaCl adjusted to pH7.4 by (0.015M-Tris–HCl buffer, before being incubated with CMP-*N*-acetyl[^{14}C]neuraminic acid and the sialyltransferase preparation. After 10min incubation the amount of labelled *N*-acetylneuraminic acid incorporated was 12.9nmol/10^9 platelets. The platelets were sedimented by centrifugation, washed twice with buffer and resuspended in the same solution. Uptake of 5-hydroxy[^{3}H]tryptamine was determined in 1ml samples of this suspension and of a similar suspension

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Table 1. *Incorporation of N-acetylneuraminic acid and uptake of 5-hydroxytryptamine by human platelets in plasma*

Time of incubation with buffer or CMP-*N*-acetyl[^{14}C]neuraminic acid (min)		*N*-Acetylneuraminic acid incorporated (nmol/10^9 platelets)	Time of incubation with 5 μM-5-hydroxy[^{3}H]tryptamine (min)	5-Hydroxytryptamine taken up (nmol/10^9 platelets)
Buffer	15	—	5	10.5
CMP-*N*-acetylneuraminic acid	15	17.8	5	18.5
Buffer	30	—	20	26
CMP-*N*-acetylneuraminic acid	30	28	20	41.2
Buffer	30	—	30	32.3
CMP-*N*-acetylneuraminic acid	40	29	30	41.6

Table 2. *Uptake of 5-hydroxytryptamine by washed control platelets and platelets enriched in N-acetylneuraminic acid*

Time of incubation with 5 μM-5-hydroxy-[^{3}H]tryptamine (min)	Uptake of 5-hydroxytryptamine (nmol/10^9 platelets)	
	Control platelets	Platelets enriched in *N*-acetylneuraminic acid
5	13	24
20	23.8	36.6
30	29.2	37.5

of control platelets that had not been incubated with CMP-*N*-acetylneuraminic acid. After incubation with 5 μM-5-hydroxy[^{3}H]tryptamine the platelets were separated from the suspending medium by high-voltage electrophoresis, centrifugation, or filtration through Millipore-filter discs (Mester, 1971); all three methods of separation yielded similar results. Platelets enriched in *N*-acetylneuraminic acid took up more 5-hydroxytryptamine than did control platelets, namely 85% more after 5min incubation, 54% more after 20min and 28% more after 30min (Table 2).

The results show that the enzymically catalysed (Mester, 1971) incorporation of *N*-acetylneuraminic acid into human platelets, whether suspended in their own citrated plasma or in buffered saline containing 0.17mM-sucrose, accelerated the uptake of 5-hydroxytryptamine. The decrease in this acceleration with time suggests that the ultimate amount of 5-hydroxytryptamine that the platelets were able to take up was not increased. In the light of what is known about the mechanism of 5-hydroxytryptamine uptake by platelets (see Born, 1970; Pletscher, 1968) the present observations may be explained by assuming that our experimental system caused additional *N*-acetylneuraminic acid to become bound to the outer platelet membrane, where the sialic acid became a component of the 5-hydroxytryptamine receptor responsible for initiating its active transport through the membrane.

We have now shown that incorporation of the same sialic acid can accelerate both reactions of platelets to 5-hydroyxtryptamine, namely its uptake and the induction of aggregation. This again raises the question whether the two reactions are mediated by the same receptor or by two different receptors. The existence of only one receptor was favoured by previous observations (Baumgartner & Born, 1968, 1969; Baumgartner, 1969). More recent evidence favoured different receptors, because 5-hydroxytryptamine uptake was inhibited by imipramine more strongly than by methysergide, whereas the reverse was true for the aggregating effect of 5-hydroxytryptamine (Born *et al.*, 1972). If there are two receptors, our latest results imply that *N*-acetylneuraminic acid is a component of both.

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