The Effect of Neuraminidase on the 5-Hydroxytryptamine Uptake of Human Platelets

Neuraminic acid occurs in glycoproteins and also in glycolipids of the platelet membrane[1,2]. It is assumed that the neuraminic acid of the platelet membrane is an essential component of the 5-hydroxytryptamine (5-HT) receptor complex. It is not yet known whether the 5-hydroxytryptamine receptor on the platelet surface is associated with neuraminic acid-containing glycoproteins of the outer cell surface or situated in interior regions of the membrane, represented there either by neuraminic acid-containing glycoproteins or neuraminic acid-containing glycolipids. Normally, in human platelets, incubated with 5-HT, the content of the amine in the platelets and also in the isolated organelles increased considerably[3]. The initial uptake of 5-HT by human platelets is linear with time in the first 30 min, reaching an equilibrium after about 90 min of incubation. In 1965 Hovig[4] showed that neuraminidase liberates neuraminic acid from platelets and that neuraminidase-treated platelets aggregate spontaneously when resuspended in plasma. Using low concentrations of neuraminidase from *Vibrio cholerae* (Behring-Werke, Marburg), 10–20 U/ml in the incubation medium and an incubation time of 10 min at 37 °C, the aggregation is greatly reduced, whereas higher amounts of the enzyme (50 U/ml) result in nearly complete clumping of the cells.

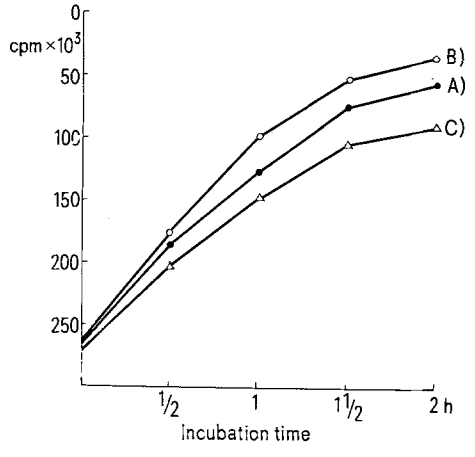

Fig. 1. Uptake of 5-hydroxy-C^{14}-tryptamine (1st preparation). A) Neuraminidase-treated platelets (pre-treated with 10 U neuraminidase/ml incubation medium). B) Neuraminidase-treated platelets (pre-treated with 20 U neuraminidase/ml incubation medium). C) Control, untreated platelets.

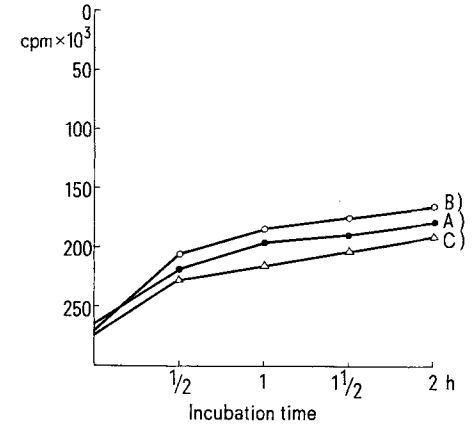

Fig. 2. Uptake of 5-hydroxy-C^{14}-tryptamine (2nd preparation). A) Neuraminidase-treated platelets (10 U enzyme/ml.) B) Neuraminidase-treated platelets (20 U enzyme/ml). C) Control, untreated platelets.

500 ml of citrated human blood was centrifuged in siliconized tubes at $700 \times g$ for 10 min at 4 °C. The platelet rich plasma was withdrawn with a siliconized pipette and then centrifuged at $2500 \times g$ for 15 min at 4 °C. The platelet layer, without the packed erythrocytes at the bottom of the centrifugation tubes, was carefully removed and resuspended in 50 ml of 0.9% NaCl buffered with sodium bicarbonate to pH 7.2 and recentrifuged at $2500 \times g$ for 15 min at 4 °C. The washing process was repeated twice. In the resulting sediment, the erythrocyte and leucocyte contamination was less than 1 per 4×10^3 platelets.

The treatment of the platelets with neuraminidase (10 U/ml and 20 U/ml respectively) was performed by suspending the platelets in 20 ml isotonic sodium acetate buffer (pH 5.5), adding the dialyzed enzyme solution and incubating at 37 °C for 10 min. Some platelet aggregations were sedimented by centrifugation at $480 \times g$ for 10 min at 4 °C. The supernatant was taken off with a siliconized pipette and centrifuged at $2500 \times g$ for 15 min at 4 °C. The sediment was washed twice with 0.9% NaCl (pH 7.2), resuspended in Ca^{2+}- and Mg^{2+}-free Krebs-Ringer bicarbonate buffer (pH 7.2) and adjusted to 5×10^8 platelets per ml. The total activity of added C^{14}-5-HT (5-hydroxy-C^{14}-tryptamine creatinine sulfate, specific activity 58 mCi/mmol, Amersham Buchler, Braunschweig) was 3×10^5 cpm. The extent of C^{14}-5-HT uptake is shown in Figure 1. The remaining activity in the medium was measured at the intervals indicated (0.5, 1, 1.5, 2 h) by removing 1 ml of the cell suspension. After centrifugation at $2500 \times g$ for 20 min at 4 °C, 0.5 ml of the supernatant was added to 10 ml of a naphthalene-dioxane scintillation solution and counted in a liquid scintillation spectrometer (Tracerlab).

The highest uptake of C^{14}-5-HT from the incubation medium was observed with platelets pre-incubated with 20 U neuraminidase/ml, a somewhat lesser uptake showed the platelets preincubated with 10 U neuraminidase/ml. The controls with untreated platelets took up the smallest amount of C^{14}-5-HT. Figure 2 represents a second platelet preparation. The same tendency is seen here, but this preparation is characterized by a lower uptake in neuraminidase-treated and untreated cells.

There seem to be individual differences in the rate of uptake, perhaps due to a different storage capacity or a different degree of saturation of the isolated platelets with 5-HT. Under identical experimental conditions used here, only a quantitative difference is obvious.

By extending the pre-incubation with neuraminidase to 30 min at 37 °C a decreased uptake of C^{14}-5-HT was generally observed. Similar results were presented re-

[1] G. A. Jamieson and D. S. Pepper, in *The Circulating Platelet* (Ed. S. A. Johnson; Academic Press, New York and London 1971), p. 189.
[2] R. V. P. Tao, C. C. Sweeley and G. A. Jamieson, J. Lipid Res. *14*, 16 (1973).
[3] M. Da Prada, J. P. Tranzer and A. Pletscher, Experientia *28*, 1328 (1972).
[4] T. Hovig, Thromb. Diath. haemorrh. *13*, 84 (1965).

cently by GLYNN[5]. He also reported that the decrease in taking up 5-HT could be partly restored by the addition of gangliosides. A similar reactivation of the 5-HT receptor complex in smooth muscle by gangliosides was described by BORN[6]. To ensure that the added C^{14}-5-HT was really transported into the platelets and not merely adsorbed to the surface of the cells, we isolated the monoamine storage organelles by the method of DA PRADA et al.[7]. About 60% of the C^{14}-5-HT content of the intact platelets could be recovered from the isolated organelles. The supernatant, after lysis of the organelles in 2 ml 1 *N* NaOH for 3 h at 25 °C, neutralization and centrifugation at 105,000 × *g* in a Beckman ultracentrifuge (Spinco L 2-50) for 30 min at 4 °C, was concentrated and analyzed on a thin-layer plate (Silica Gel H, Merck, Darmstadt) in the developing system: methylacetate-isopropanol-25% NH_4OH (9:7:4)[8]. The radioactive spots were localized with a Berthold thin-layer scanner, model LB 2722.

Our results indicate that the neuraminic acid of the outer layer of the platelet surface membrane, which is easily released by neuraminidase, is probably not involved in the 5-hydroxytryptamine receptor. It seems that the partial loss of the peripherical neuraminic acid, and the resulting decrease of the negative charge of the platelet surface, facilitates the taking up of 5-HT.

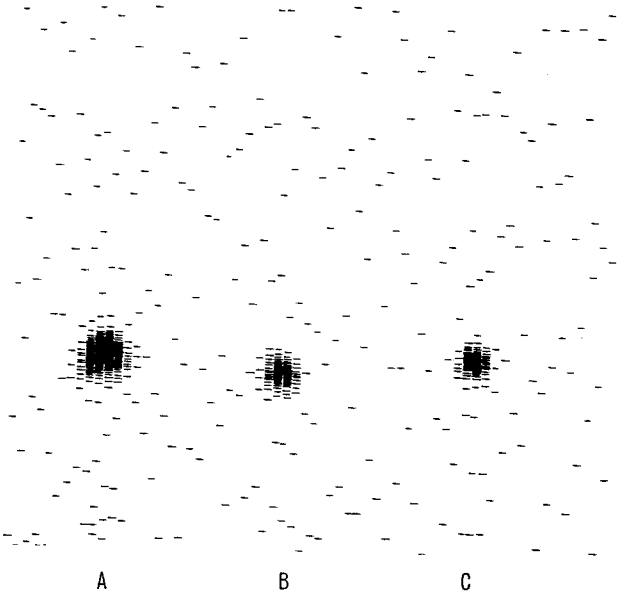

Fig. 3. Radio thin-layer chromatogram of C^{14}-5-HT. A) Test: C^{14}-5-HT. B) C^{14}-5-HT, lysed from isolated platelets. C) C^{14}-5-HT, lysed from isolated storage organelles.

The increase of the negative charge on the platelet membrane, by additional incorporation of neuraminic acid transfered by a sialyltransferase from rat liver homogenate, resulted in a reduction of the uptake of potassium ions[9]. The addition of 5-HT always enhanced the taking up of K^+ markedly. From these results it is highly evident that the extent of taking up K^+ by platelets is charge dependent, and that the diminution of the negative charge on the platelet surface by short neuraminidase treatment should accelerate the K^+-uptake into the platelets, too.

In our experiments, with low neuraminidase concentrations and a short incubation time, the neuraminidase of *Vibrio cholerae* did not liberate the neuraminic acid of glycolipids in the interior regions of the platelet membrane. The complete ganglioside pattern could be extracted after this treatment, but also neuraminic acid-containing glycoproteins were still present.

From the results after prolonged incubation with neuraminidase, it can be concluded that the recognition site, the absorption and the active transport mechanism of 5-HT is located within the platelet membrane and that the neuraminic acid, bound to glycoproteins or glycolipids within the membrane, participates in this process.

Zusammenfassung. Am 5-Hydroxytryptaminrezeptor der Thrombozyten ist die periphere Glykoprotein-Neuraminsäure nicht beteiligt. Der Verlust dieser peripheren Glykoprotein-Neuraminsäure fördert den Einstrom von 5-HT in die Thrombozyten. Der Rezeptor und der Transportmechanismus werden im Innern der Membran vermutet. Sie sind durch kurzfristige Neuraminidaseeinwirkung nicht erreichbar.

W. GIELEN[10] and B. VIEHÖFER

Institute of Pharmacology, University of Cologne, Gleueler Str. 24, D-5 Köln 41 (Federal Republic of Germany), 21 March 1974.

[5] M. F. X. GLYNN, Am. J. clin. Path. *60*, 636 (1973).
[6] G. V. R. BORN, in *Smooth Muscle* (Eds. E. BÜLBRING, A. F. BRADING, A. W. JONES and T. TOMITA; Edward Arnold Publishers Ltd., London 1970), p. 418.
[7] M. DA PRADA, K. VON BERLEPSCH and A. PLETSCHER, Naunyn-Schmiedebergs Arch. Pharmac. *275*, 315 (1972).
[8] E. STAHL and H. KALDEWEY, Hoppe-Seyler's Z. physiol. Chem. *323*, 182 (1961).
[9] F. MICHAL, G. V. R. BORN, L. MESTER and L. SZABADOS, Biochem. J. *129*, 977 (1972).
[10] We gratefully acknowledge the support of this study by the Deutsche Forschungsgemeinschaft and the Fonds der chemischen Industrie.

The Inhibitory Effect of Toluidine Blue and Methylene Blue on Rat Mast Cell Damage by Promethazine, Chlorpromethazine and Chlorpromazine. Its Reversal by Glucose

In a previous paper it was shown that phenothiazine stains prevent rat mesentery mast cell damage induced by compound 48/80 and by the antihistamine drugs, diphenhydramine and chlorcylizine. It was also shown that this blocking effect was similar to that of metabolic inhibitors[1]. The present report describes the inhibitory effect of toluidine blue and methylene blue on rat mesentery mast cell damage by phenothiazine compounds with antihistamine activity: promethazine, chlorpromethazine, and chlorpromazine. The anti-enzymatic mechanism postulated to explain the inhibitory effect of these stains is substantiated.

[1] I. VUGMAN and M. L. M. DO PRADO, Naunyn Schmiedebergs Arch. Pharmac. *279*, 173 (1973).