Br. J. Pharmac. (1979), **65**, 557–563

EFFECTS OF CONCANAVALIN A ON 5-HYDROXYTRYPTAMINE UPTAKE BY RABBIT BLOOD PLATELETS AND ON THEIR ULTRASTRUCTURE

H. NISHIO, T. SEGAWA & H. TAKAGI*

Department of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734 and *Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan

1 Effects of concanavalin A (Con A) and other lectins on 5-hydroxytryptamine (5-HT) uptake by rabbit blood platelets and on their ultrastructure were studied.

2 Uptake of [^{3}H]-5-HT by platelets was decreased by application of Con A, E-PHA (lectin from *Phaseolus vulgaris*) and lentil-PHA (lectin from *Lens culinaris*), but not by wheat germ agglutinin (WGA). Con A induced specific changes in the ultrastructure of platelets, causing (i) a change in external appearance from a discoid to an irregularly spherical shape, (ii) re-arrangement of the canalicular system and formation of a concentric structure. These effects of Con A on platelets were antagonized by pretreatment with α-methyl-D-mannoside (α-MM), a specific inhibitor of Con A binding to glycoprotein.

3 The inhibition of 5-HT uptake by Con A was antagonized by colchicine, vinblastine and sodium nitroprusside (SNP), but not by cytochalasin B.

4 Theophylline, papaverine and dibutyryl cyclic adenosine 3′,5′-monophosphate (db cyclic AMP) antagonized the effect of Con A on 5-HT uptake, but dibutyryl cyclic guanosine 3′,5′-monophosphate had no effect. Theophylline and db cyclic AMP did not influence the effect of Con A on the ultrastructure of platelets.

5 It is suggested that binding of Con A to specific receptor glycoproteins can inhibit the 5-HT uptake system of platelets. Microtubules, contractile protein and the membrane adenylate cyclase system of platelets may also be regulatory factors in this mechanism.

Introduction

Lectins, isolated from a wide variety of plants and animals, bind specifically to carbohydrate and glycoprotein, and have various physiological effects (e.g. agglutination of erythrocytes with clinical blood type specificity, modification of lymphoid cell function, transformed cell specific agglutination) hence their value as research tools for exploring carbohydrate grouping on the cell surface have been well discussed (Lis & Sharon, 1973; Nicolson, 1974). Recent studies have shown that three major glycoproteins are exposed on the surface of platelets (Phillips, 1972), and their binding with lectin molecules results in aggregation of platelets (Majerus & Brodie, 1972; Patscheke, Brossmer & Wörner, 1977).

In the present study, the effects of lectins on 5-hydroxytryptamine (5-HT) uptake by platelets and on their ultrastructure were investigated. Evidence is presented for a close relationship between the 5-HT uptake system and lectin receptors on the membrane of platelets.

Methods

Rabbits of either sex weighing 2.0 to 2.5 kg were used. Whole blood was collected from the carotid artery, mixed with 1/10 volume of 3.8% sodium citrate and centrifuged at 150 *g* for 20 min at room temperature. The supernatants (15 ml) were diluted with 35 ml of buffered salt solution (BSS) (composition mM: NaCl 134, $MgCl_2$ 3, D-glucose 5, Tris-HCl buffer pH 7.4, 15), to which heparin solution was added to yield a final concentration of 10 units/ml.

The final dilution of platelet-rich plasma was separated into 1 ml aliquots, each of which was transferred to a test tube containing 0.4 ml of BSS or of the

0007-1188/79/040557-07 $01.00

drug solution to be tested. After preincubation for 30 min at 37°C, [^{3}H]-5-HT (1.27×10^{-7} M) was added to the samples and the mixtures were further incubated for 3 min. The incubation was terminated by addition of 3 ml ice cold BSS and the mixtures were then centrifuged at 1500 *g* for 30 min at 4°C. The platelets thus sedimented were washed twice with ice cold BSS. After draining, the radioactivity of the platelets was determined. Blank values were obtained from the samples to which the radioactive material was added after the test tubes had been placed in ice water. At the same time, the 5-HT concentration of the platelets was determined fluorometrically by the method of Curzon & Green (1970).

The preparation of samples for scanning electron microscopy was carried out by a modification of the method of Sanders, Alexander & Braylon (1975). The samples in suspension were fixed by adding at least 10 volumes of 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 h at 37°C; the temperature was then reduced to 4°C for 24 h. The platelet suspension was dropped onto a glass slide previously coated with poly-L-lysine and this was left in a moist chamber at 4°C for 12 h after which the platelets adhered to the glass slide. After dehydration in a series of graded alcohols and drying with CO_2 using a Hitachi HCP-1 critical point drying apparatus, the glass slide was coated with gold in an Eiko 1B-I ion cleaner. A JSM-U3 scanning electron microscope was used to examine the sample.

Platelet samples for transmission electron microscopy were prepared by a method based on that of White (1968). The cell suspension was fixed at 37°C for 1 h after mixing with an equal volume of 4% glutaraldehyde in the phosphate buffer. The mixtures were then centrifuged at room temperature to obtain platelet buttons. Supernatant plasma and fixative were discarded and 3 to 4 ml of fresh 2% glutaraldehyde in phosphate buffer was layered over the buttons. After hardening at 4°C, the buttons were cut into small pieces and dropped into chilled 1% osmium tetroxide in the phosphate buffer. After 1 h at 4°C, the samples were dehydrated in a series of graded alcohols and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate, and examined under a Hitachi HS-7A electron microscope.

Drugs

The following drugs were used: 5-hydroxy[G-^{3}H]-tryptamine creatinine sulphate (Radiochemical Centre, Amersham); concanavalin A (Sigma Chemical Co., Saint-Louis, Mo.); E-PHA (Difco Laboratories, Detroit, Mich.); lentil PHA, wheat germ agglutinin (WGA), dibutyryl cyclic adenosine 3′,5′-monophosphate (db cyclic AMP) and dibytyryl cyclic guanosine 3′,5′-monophosphate (db cyclic GMP) (Boehringer, Mannheim, West Germany); colchicine (donated by Shionogi Pharmaceutical Co., Ltd., Osaka, Japan); vinblastine (donated by Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan); cytochalasin B (Aldrich, Milwaukee, Wis.). All other chemicals were of analytical grade.

Results

Effects of concanavalin A on [^{3}H]-5-hydroxytryptamine uptake by platelets and on platelet ultrastructure

Con A (1.35 to 135 μg/ml) had no effect on endogenous 5-HT concentration of platelets, but was a potent inhibitor of [^{3}H]-5-HT uptake at a concentration of 135 μg/ml (Figure 1). This inhibitory effect was evident only after Con A had been preincubated with the platelets for 20 min (Figure 2). α-Methyl-D-mannoside (α-MM, 5×10^{-3} to 5×10^{-2} M), a competitive inhibitor of Con A binding to sugar residue, almost completely antagonized the inhibitory effect of Con A on 5-HT uptake (Figure 3).

Con A induced marked changes in ultrastructure of platelets as observed with the transmission and scanning electron microscope. Thus, the discoid appearance of intact platelets changed to an irregular spherical shape and there was a re-arrangement of the canalicular system to form a concentric structure. All of these effects of Con A on platelets were antagonized by pretreatment with α-MM (Figures 4, 5).

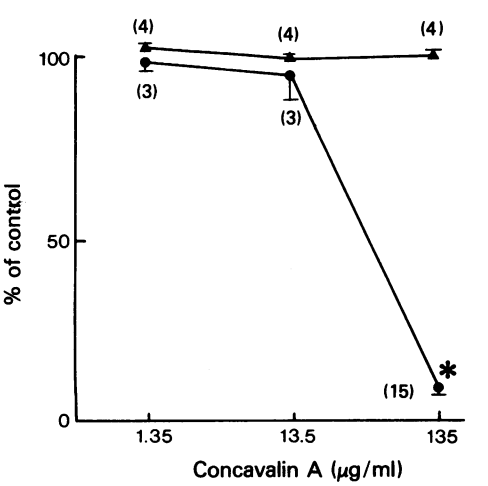

Figure 1 Effect of concanavalin A on 5-hydroxytryptamine (5-HT) transport in platelets: (▲) endogenous 5-HT concentration; (●) [^{3}H]-5-HT uptake. Values are the mean of the number of experiments indicated in parentheses, vertical bars are s.e. mean. *Significantly different from control, $P < 0.001$.

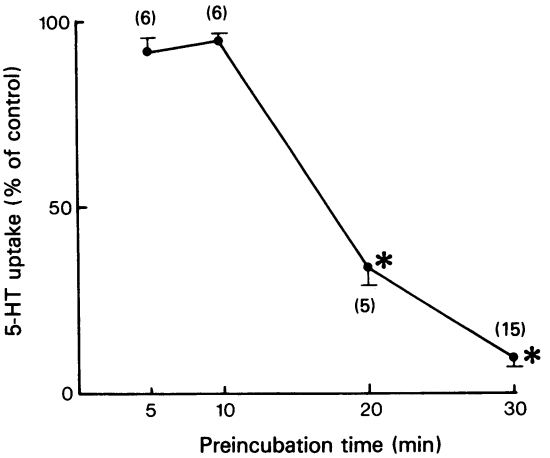

Figure 2 Preincubation time course of the effect of concanavalin A on 5-hydroxytryptamine (5-HT) uptake by platelets. Values are the mean of the number of experiments indicated in parentheses, vertical bars are s.e. mean. *Significantly different from control, $P < 0.001$.

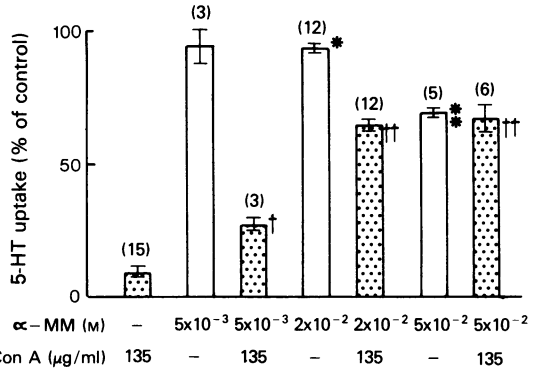

Figure 3 Antagonistic effect of α-methyl-D-mannoside (α-MM) on 5-hydroxytryptamine (5-HT) uptake inhibition by concanavalin A (Con A). Values are the mean of the number of experiments indicated in parentheses, vertical bars are s.e. mean. Significantly different from control, $*P < 0.01$; $**P < 0.001$; $\dagger P < 0.005$; $\dagger\dagger P < 0.001$. †, ††; vs Con A alone.

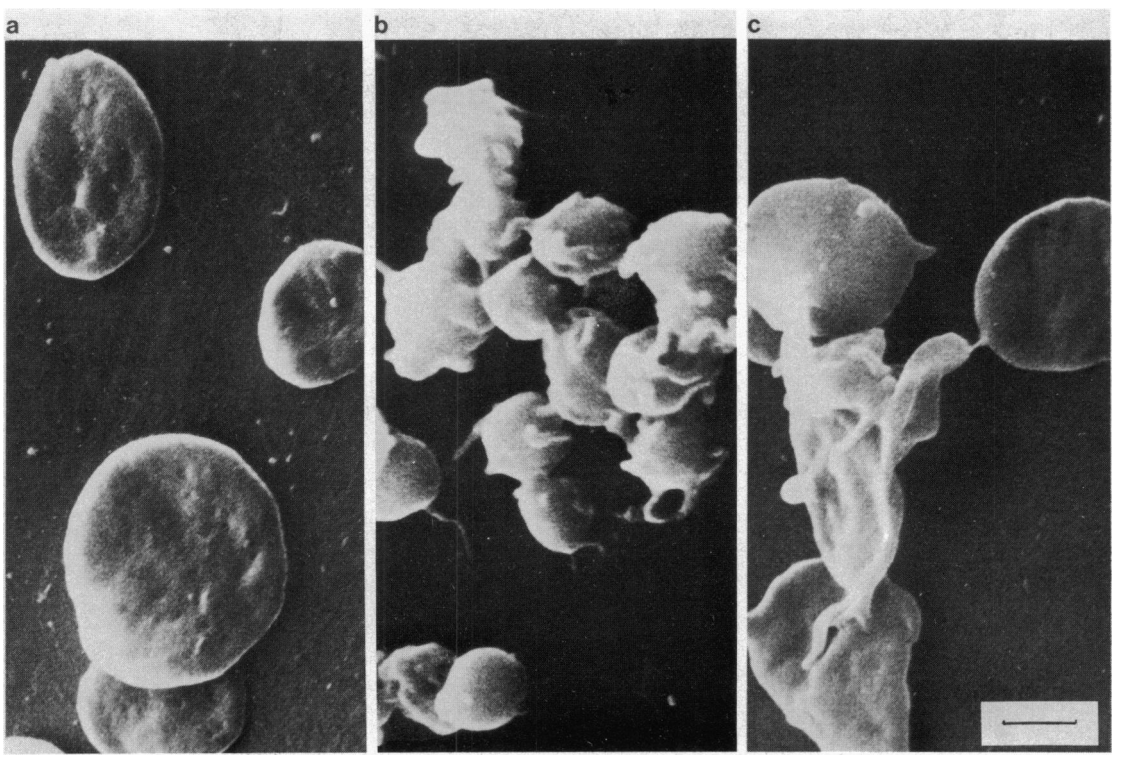

Figure 4 Scanning electronmicrograph of concanavalin A (Con A) and α-methyl-D-mannoside (α-MM) treated platelets. (a) Intact platelets: Note the discoid appearance of platelets and the open canalicular system. (b) Con A treatment: Con A (135 μg/ml, 30 min)-treated platelets have an irregular spherical appearance. (c) Con A and α-MM treatment: the effect of Con A on ultrastructure of the platelets is antagonized by α-MM (2×10^{-2} M) pretreatment. Scale; 1.0 μm.

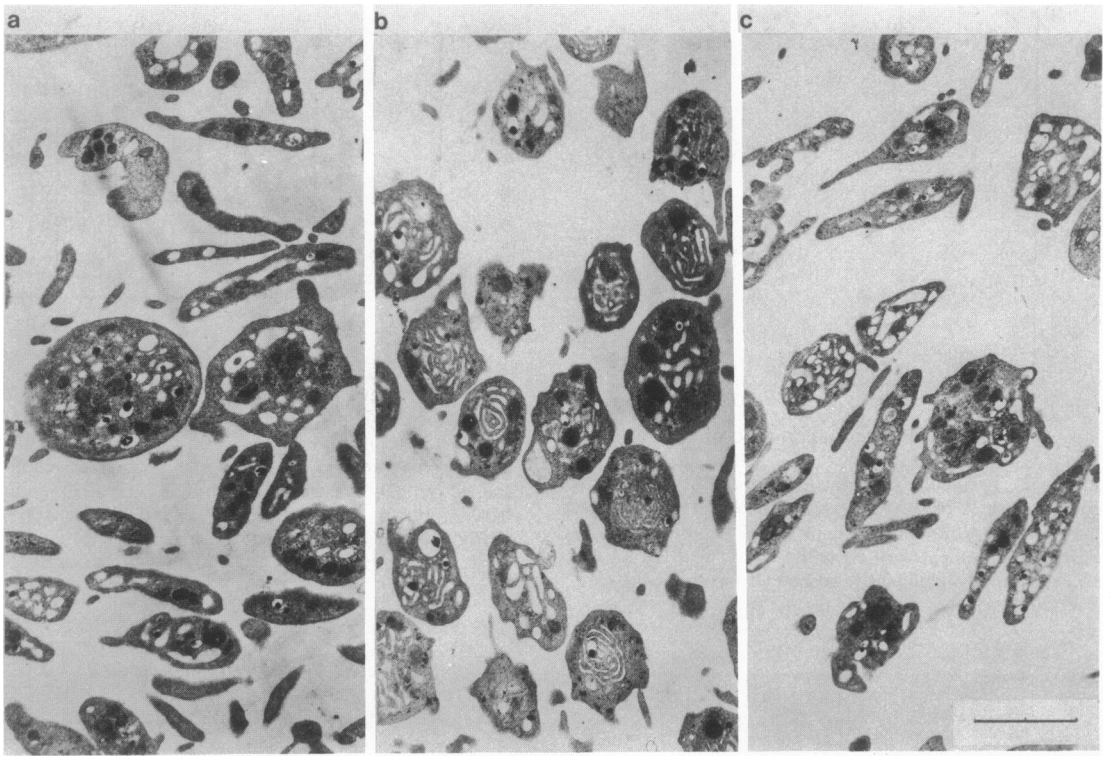

Figure 5 Transmission electronmicrograph of concanavalin A (Con A) and α-methyl-D-mannoside (α-MM) treated platelets. (a) Intact platelets: note the vacuole-like canalicular system. (b) Con A treatment: Con A (135 μg/ml, 30 min) induced striking changes, particularly in the canalicular system where a concentric structure was formed. (c) Con A and α-MM treatment: the effect of Con A on ultrastructure of the platelets is antagonized by α-MM (2×10^{-2} M) pretreatment. Scale; 2.0 μm.

Table 1 Concanavalin A (Con A) inhibition of 5-hydroxytryptamine (5-HT) uptake by rabbit blood platelets: effect of drugs

Compound (M)[1]	*% inhibition of 5-HT uptake*[2] *by Con A*
—	91.0 ± 2.0(15)
Colchicine (5×10^{-4})	46.1 ± 8.1***(6)
Vinblastine (10^{-5})	50.0 ± 8.2***(8)
Cytochalasin B (5×10^{-5})	98.7 ± 0.9(3)
Sodium nitroprusside (10^{-4})	45.5 ± 9.5***(6)
Theophylline (10^{-3})	12.9 ± 3.3***(6)
Papaverine (2×10^{-5})	50.4 ± 13.0***(9)
Db cyclic AMP (5×10^{-4})	15.5 ± 4.9***(6)
Db cyclic GMP (10^{-3})	91.5 ± 1.9 (3)

[1]Blood platelets were pre-incubated with the drug for 30 min.
[2]Inhibitory effect of Con A (135 μg/ml) on 5-HT uptake was determined. The values shown are the mean ±s.e.mean. The number of experiments is given in parentheses.
*** Significantly different from control, $P < 0.001$.

Effects of colchicine, vinblastine, cytochalasin B and sodium nitroprusside on [^{3}H]-5-hydroxytryptamine uptake inhibition by concanavalin A

Both colchicine (5×10^{-4} M) and vinblastine (10^{-5} M), which affected the microtubules of platelets to the point of disappearance, had no effect on [^{3}H]-5-HT uptake activity by platelets. However, these agents significantly antagonized the inhibitory effect of Con A on 5-HT uptake. On the other hand, cytochalasin B, which affected the microfilaments of platelets, did not antagonize the inhibitory effect of Con A on 5-HT uptake but tended to enhance the inhibitory effect of Con A on 5-HT uptake (Table 1).

SNP, which was reported to affect thrombostenin and influence platelet function (Saxon & Kattlove, 1976), had no effect on the [^{3}H]-5-HT uptake of platelets, but did significantly antagonize the inhibitory effect of Con A on 5-HT uptake (Table 1).

Effects of theophylline and papaverine on [^{3}H]-5-hydroxytryptamine uptake inhibition by concanavalin A

The effects of theophylline and papaverine, which inhibit cyclic nucleotide phosphodiesterase and influence platelet function (Salzman & Weisenberger, 1972) on [^{3}H]-5-HT uptake inhibition by Con A were studied. Theophylline (10^{-3} M) almost completely antagonized the inhibitory effect of Con A on 5-HT uptake. Similarly, the 5-HT uptake inhibition of Con A was significantly antagonized by papaverine (2×10^{-5} M) pretreatment (Table 1). Theophylline did not antagonize the effect of Con A on the ultrastructure of platelets (Figure 6).

Effects of dibutyryl cyclic AMP and dibutyryl cyclic GMP on [^{3}H]-5-hydroxytryptamine uptake inhibition by concanavalin A

Attempts were also made to clarify the involvement of cyclic nucleotide in [^{3}H]-5-HT uptake inhibition by Con A, with db cyclic AMP and db cyclic GMP. Db cyclic AMP (5×10^{-4} M) almost completely antagonized the inhibitory effect of Con A, while db cyclic GMP had no effect at concentrations up to 10^{-3} M (Table 1).

Effects of E-PHA, lentil PHA and wheat germ agglutinin on [^{3}H]-5-hydroxytryptamine uptake by platelets

Lentil PHA, which has the same sugar specificity as Con A, had a strong inhibitory effect on 5-HT uptake. Similarly, E-PHA, which binds specifically to the sugar, N-acetyl-D-galactosamine, had an inhibitory effect on 5-HT uptake by platelets. On the other hand, WGA, which binds specifically to the sugar, N-acetylchitobiose, had no effect on 5-HT uptake by platelets (Table 2).

Discussion

It is generally accepted that binding of lectins to human blood platelets results in aggregation, release reaction and inhibition of adenylate cyclase (Cooper, Mason & Brinkhous, 1976). Lectins have various sugar specificities depending on their origin. Greenberg & Jamieson (1974) reported the effect of nine kinds of lectins on aggregation, and the ADP- and 5-HT-induced release reaction of human blood platelets. They found that various effects of lectins on platelet functions were dependent on the sugar specificity. But as reported by Jones & Evans (1976), there were discrepancies among investigators in the results with the same kind of lectin. For example, Feagler, Tillack, Chaplin & Majerus (1974) found that lentil PHA bound to human blood platelet membrane without inducing either aggregation or release reaction. This result was contrary to the findings of Greenberg & Jamieson (1974) who showed that lentil PHA induced a 5-HT release reaction. In our experiments with rabbit blood platelets, we found the opposite results with respect to release of endogenous 5-HT. These discrepancies might be due to differences in the species of blood donor, purity of the lectins and other experimental conditions.

Con A, the sugar specificity of which was α-D-mannopyranose and α-D-glucopyranose (Lis & Sharon, 1973), did not induce 5-HT release, instead [^{3}H]-5-HT uptake was inhibited and the platelet ultrastructure was changed. E-PHA, which binds specifically to the sugar, N-acetyl-D-galactosamine, had the same inhibitory effect as Con A on 5-HT uptake. On the other hand, WGA, which binds specifically to N-acetylchitobiose, had no effect on 5-HT uptake. These results indicate that lectin receptors on the surface of the platelet membrane play a regulatory role in the 5-HT carrier system and that binding of lectin molecules to this receptor leads to inhibition of 5-HT uptake by means of conformational changes in the membrane. These speculations were further supported by the fact that the effect of Con A on platelets was antagonized by α-MM, a competitive inhibitor of Con A binding to the receptor.

The ultrastructural changes seen with Con A application were also antagonized by α-MM. But theophylline, which almost completely antagonized the inhibitory effect of Con A on 5-HT uptake, had no effect on the ultrastructural changes caused by Con A. These results indicate that the inhibitory effect of Con A on 5-HT uptake was not merely secondary to changes in the ultrastructure of the platelets.

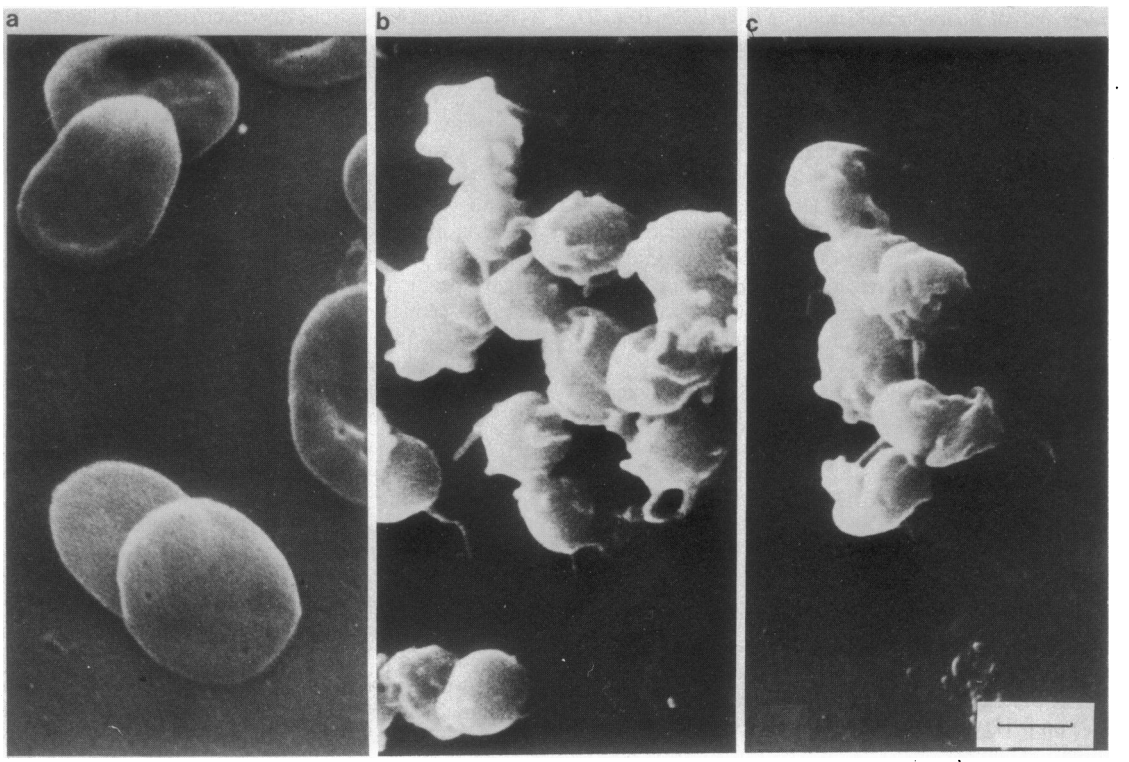

Figure 6 Scanning electronmicrograph of concanavalin A (Con A)-and theophylline-treated platelets. (a) Theophylline treatment: there is no appreciable change compared with intact platelets. (b) Con A treatment: Con A (135 μg/ml, 30 min)-treated platelets have an irregular spherical appearance. (c) Con A and theophylline treatment: theophylline (10^{-3} M) did not antagonize the effect of Con A on ultrastructure of the platelets. Scale; 1.0 μm.

Table 2 Effect of various lectins on 5-hydroxytryptamine (5-HT) uptake by rabbit blood platelets

Compound (μg/ml)[1]		*% of control*[2] *5-HT uptake*	*% of control*[2] *endogenous 5-HT concentration*
	1.5	97.0 ± 4.2(6)	99.1 ± 2.8(6)
E-PHA	15.0	94.6 ± 4.1(6)	100.6 ± 1.7(6)
	150.0	27.6 ± 5.2****(10)	88.2 ± 6.5(6)
	0.1	88.7 ± 3.5*(6)	99.0 ± 2.8(6)
Lentil-PHA	1.0	65.4 ± 6.9***(7)	101.9 ± 4.1(6)
	10.0	22.8 ± 6.0****(9)	107.2 ± 1.5**(5)
	1.0	95.5 ± 3.0(6)	—
WGA	10.0	94.5 ± 4.2(9)	—
	100.0	102.0 ± 1.4(9)	103.6 ± 3.7(5)

[1]Blood platelets were incubated with lectin for 30 min. [2]The control values are referred to as 100%. The values shown are the mean ± s.e.mean. The number of experiments is given in parentheses. E-PHA: lectin from *Phaseolus vulgaris*; Lentil PHA: lectin from *Lens clinaris*; WGA: wheat germ agglutinin.
Significantly different from control, *$P < 0.05$ **$P < 0.01$ ***$P < 0.005$
****$P < 0.001$.

Yahara & Edelman (1973) who demonstrated a relationship between Con A effect and microtubules using lymphocytes, showed that microtubule dissociating reagents, such as colchicine and vinblastine, antagonized the effect of Con A and that the integrity of the membrane structure related to microtubules was required for the effect of Con A.

The effect of Con A on 5-HT uptake by platelets was also antagonized by a microtubule dissociating reagent and by an inhibitor of the contractile protein system such as SNP. Cytochalasin B, which has an effect on microfilaments, tended to enhance the effect of Con A. These results suggest that the integrity of membrane-associated components such as microtubules and the contractile protein system are required for the effect of Con A on 5-HT uptake.

As to adenylate cyclase inhibition of platelets by lectin, Tollefsen, Feagler & Majerus (1974) speculated that this inhibition was one of the steps of the platelet releasing reaction by lectin (E-PHA). They found that E-PHA, which induced endogenous 5-HT release from human blood platelets, inhibited adenylate cyclase of platelets, and that reagents such as theophylline, db cyclic AMP and prostaglandin E_1 (PGE_1) which elevate intracellular cyclic AMP concentration of platelets, counteract the effect of E-PHA. Similarly, Schmukler, Zieve & Jewett (1976) found that the release of 5-HT from human blood platelets by Con A was antagonized by PGE_1 treatment.

Our results indicate that there is a close relationship between the effect of Con A and the cyclic AMP system. We also found that theophylline had a stimulatory effect on 5-HT uptake by platelets and that this effect was potentiated by PGE_1 (Nishio, Segawa & Takagi, 1976). From these results it is suggested that adenylate cyclase is involved in the 5-HT uptake system of platelets and that inhibition by lectin may indeed be one of the steps involved in the inhibition of 5-HT uptake.

Our thanks are due to Miss S. Kikuchi and Mr Y. Okuma for technical assistance and M. Ohara, Kyoto University, for assistance with the manuscript. This work was supported in part by grant No. 277656 for scientific research from the Ministry of Education, Science and Culture, Japan.

References

COOPER, H.A., MASON, R.G & BRINKHOUS, K.M. (1976). The platelet: Membrane and surface reaction. *A. Rev. Physiol.*, **38**, 501–535.

CURZON, G. & GREEN, A.R. (1970). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Br. J. Pharmac.*, **39**, 635–655.

FEAGLER, J.R., TILLACK, T.W., CHAPLIN, D.D. & MAJERUS, P.W. (1974). The effects of thrombin on phytohemagglutinin receptor sites in human platelets. *J. cell Biol.*, **60**, 541–553.

GREENBERG, J.H. & JAMIESON, G.A. (1974). The effect of various lectins on platelet aggregation and release. *Biochem. biophys. Acta*, **345**, 231–242.

JONES, B. & EVANS, P.M. (1976). On the susceptibility of human platelets to aggregation by concanavalin A and the effect of this lectin on their response to ADP. *Biochem. biophys. Acta*, **448**, 368–378.

LIS, H. & SHARON, N. (1973). The biochemistry of plant lectins (phytohemagglutinins). *Ann. Rev. Biochem.*, **42**, 541–574.

MAJERUS, P.W. & BRODIE, G.N. (1972). The binding of phytohemagglutinins to human platelet plasma membranes. *J. biol. Chem.*, **247**, 4253–4257.

NICOLSON, G.L. (1974). The interaction of lectins with animal cell surfaces. *Int. Rev. Cytol.*, **39**, 89–190.

NISHIO, H., SEGAWA, T. & TAKAGI, H. (1976). Influence of cold treatment and cyclic AMP on the 5-HT uptake by rabbit blood platelets. *Jap. J. Pharmac.*, **26**, 52P.

PATSCHEKE, H., BROSSMER, R. & WÖRNER, P. (1977). D-galactose-binding lectins induced a differential response of blood platelets. *Biochem. biophys. Res. Commun.*, **75**, 200–206.

PHILLIPS, D.R. (1972). Effect of trypsin on the exposed polypeptide and glycoproteins in the human platelet membrane. *Biochemistry*, **11**, 4582–4588.

SALZMAN, E.W. & WEISENBERGER, H. (1972). Role of cyclic AMP in platelet function. In *Advances in Cyclic Nucleotide Research*. ed. Greengard, P. & Robison, G.A. vol. 1, pp. 231–247. New York: Raven Press.

SANDERS, S.K., ALEXANDER, E.L. & BRAYLON, R.C. (1975). A high-yield technique for preparing cells fixed in suspension for scanning electron microscopy. *J. cell Biol.*, **67**, 476–480.

SAXON, A. & KATTLOVE, H.E. (1976). Platelet inhibition by sodium nitroprusside, a smooth muscle inhibitor. *Blood*, **47**, 957–961.

SCHMUKLER, M., ZIEVE, P.D. & JEWETT, P.B. (1976). Induction of the platelet release reaction by concanavalin A—relation of Con A binding to release and modification of release by ATP, PGE_1 and amantadine. *Biochem. Pharmac.*, **25**, 1819–1824.

TOLLEFSEN, D.M., FEAGLER, J.R. & MAJERUS, P.W. (1974). Induction of the platelet release reaction by phytohemagglutinin. *J. clin. Invest.*, **53**, 211–218.

WHITE, J.G. (1968). Tubular elements in platelet granules. *Blood*, **32**, 148–156.

YAHARA, I. & EDELMAN, G.M. (1973). Modulation of lymphocyte receptor redistribution by concanavalin A, anti-mitotic agents and alterations of pH. *Nature*, **246**, 152–155.

(Received May 24, 1978.
Revised September 27, 1978.)