Effect of Concanavalin A on 5'-Nucleotidase Activity of Rabbit Blood Platelets

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Abstract—Ecto-5'-nucleotidase (ecto-5'-NU) of platelets was enhanced by concanavalin A (Con A). This effect of Con A was antagonized by α-methyl-D-mannose, a specific antagonist of Con A binding to glycoprotein. Coformycin, an adenosine deaminase inhibitor, did not change the effect of Con A on the ecto-5'-NU. Uptake of adenosine by platelets was not affected by Con A. It was suggested that the ecto-5'-NU of platelet might be a direct and primary site of action of Con A.

Concanavalin A (Con A), a lectin from Canavalia ensiformis, binds specifically to sugars with D-arabinose configuration like D-mannose or D-glucose and membrane glycoproteins containing such a sugar residue. Con A binding on the plasma membrane of various cell types induced changes in their biological or biochemical properties (1). In our previous studies, Con A was shown to have a potent inhibitory effect on the uptake of 5-hydroxytryptamine (5-HT) by blood platelets (2), and involvement of the adenylate cyclase system of platelets in the effect of Con A was suggested (3). It was postulated that Con A might exert its inhibitory effect on the 5-HT uptake through the inhibition of adenylate cyclase activity (3). However, mechanism of the effect of Con A on adenylate cyclase of blood platelet has not been clearly elucidated.

5'-Nucleotidase (EC 3.1.3.5) is an ectoenzyme which specifically hydrolyzes nucleoside 5'-monophosphates to nucleosides and Pi. Slavik et al. (4) and Dornand et al. (5) demonstrated that lymphocyte 5'-nucleotidase, which was shown to be inhibited by Con A, is one of the lectin receptors. They suggested that Con A might indirectly control cyclic AMP level through the level of adenosine (6).

In the present work, the effect of Con A on ecto-5'-nucleotidase activity of platelets and the effect on uptake of adenosine, the end-product of ecto-5'-nucleotidase, by platelets were investigated to determine if there is a possible involvement of this enzyme activity in the effect of Con A on blood platelets (2, 3).

\[2\text{-}^3\text{H}]\text{Adenosine} \ 5'\text{-monophosphate ammonium salt (}^3\text{H}-\text{AMP, 11.7 Ci/mmol)} \] and \[2\text{-}^3\text{H}]\text{adenosine (}^3\text{H}-\text{adenosine, 24 Ci/mmol)} \] were obtained from Amersham. Concanavalin A (Con A) from Boehringer, coformycin from Behring-Diagnostics, diprydamole from Sigma. Whole blood was collected from the carotid artery of rabbits, mixed with 1/10 volume of 3.8% sodium citrate and centrifuged at 150xg for 20 min at room temperature. The supernatant (platelet-rich plasma: PRP) was collected and diluted with buffered salt solution (BSS: 134 mM NaCl, 3 mM MgCl₂, 5 mM D-glucose, 15 mM Tris-Cl buffer, pH 7.4) to make diluted PRP (ca. 7x10⁹ platelets/ml). 5'-Nucleotidase activity was assayed by a modification of the method of Suran (7). The incubation mixture consisted of 300 µl of the diluted PRP, 10 µl of 100 mM AMP, 10 µl of 3H-AMP (0.63 µCi/ml), and 180 µl TM-buffer (4 mM MgCl₂, 40 mM Tris-Cl buffer pH 7.4) containing test substances. Diprydamole (50 µM) was included in the...
incubation medium to prevent conversion of the product by the uptake into platelets (8, 9). The mixture was incubated at 37°C for 20 min. The reaction was stopped by adding 100 μl of 4 mM adenosine and placing the tubes in boiling water for 3 min. The mixture was then centrifuged at 10,000×g for 10 min. 3H-Adenosine, the end-product, in the supernatant was purified with an anion exchange resin column (Bio-Rad, AG 1-X4, 200–400 mesh) packed in a tuberculin syringe (2.3 cm), and the radioactivity was counted. The results were expressed as the percent activity of the control experiment. The uptake of 3H-adenosine was measured as follows: One ml aliquots of the diluted PRP was transferred into a polypropylene test tube containing 0.2 ml BSS with test substance. After pre-incubation in the presence of 10 units/ml heparin, 3H-adenosine (1.04 μM) was added to the sample, and the mixtures were further incubated for 5 min. The incubation was terminated by adding 3 ml of ice cold BSS, and the mixtures were centrifuged at 1,500×g for 30 min at 4°C. Platelets thus sedimented were washed twice with ice cold BSS. The radioactivity in the platelets was counted. The results were expressed as the percent activity of the control experiment and were statistically analyzed by Student's t-test.

Ecto-5'-NU activity of blood platelets was assayed using intact blood platelets, in the presence of dipyridamole, an uptake inhibitor of the product into platelets. This enzyme activity was not affected by coformycin (Fig. 1), indicating that there was no involvement of adenosine metabolism in this assay system. Con A dose-dependently potentiated both enzyme activity with and without coformycin. This effect of Con A was antagonized by α-methyl-D-mannoside, a specific antagonist of Con A binding to glycoprotein (2, 4), suggesting Con A binding to the surface glycoprotein residues, of platelets is required for its effect. As shown in Fig. 2, Con A had no influence on the uptake of 3H-adenosine. Also, it was shown that the uptake of 3H-adenosine was not affected by coformycin (Fig. 2), indicating that the deamination of adenosine does not serve as the inactivating process for adenosine outside of the platelets.

Jakobs et al. (10) reported the regulation of platelet adenylate cyclase by adenosine. They suggested that the elevation of adenosine concentration might result in the

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**Fig. 1.** Effect of concanavalin A (5×10⁻⁵–2×10⁻⁴ g/ml) on ecto-5'-nucleotidase of the intact blood platelet with or without coformycin (1.5×10⁻⁸ M) or α-methyl-D-mannoside (α-MM, 2×10⁻² M). The mean activity in the control sample (without Con A, coformycin and α-MM) was 0.600±0.084 nmole adenosine formed/10⁹ platelets/20 min (n=20). Mean values±S.E.M. for 4 experiments. *Significant difference at P<0.05 (vs. control). *Significant difference at P<0.05 (vs. coformycin alone).
inhibition of the adenylate cyclase (10). Our present results indicate that ecto-5'-NU, which has been shown to have a glycoprotein moiety (11), might be a direct site of action for the inhibitory effect of Con A on adenylate cyclase (3) and subsequently on 5-HT uptake (2). Con A might serve as the 5-HT uptake inhibitor (2) through the activation of ecto-5'-NU, which results in the local elevation of adenosine concentration and the inhibition of adenylate cyclase. Adenosine might serve as a transmembrane modulator of 5-HT uptake system through the inhibition of adenylate cyclase in the platelet (3).

It is very interesting to note that opposite relationships to ours between Con A and 5'-NU activity were observed in lymphocytes (12), liver cells (13) and Schwann cells (14). It was described that the ecto-5'-NU activity of these cells were not potentiated, but inhibited by Con A. The effect of Con A on the function of lymphocytes was also speculated to be mediated through its direct effect on ecto-5'-NU (11). Our present paper is the first report that clearly demonstrates the stimulatory action of Con A of 5'-NU.

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