CONCANAVALIN A INHIBITION OF LIVER PLASMA MEMBRANE-BOUND CYCLIC AMP-PHOSPHODIESTERASE

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1. Introduction

Concanavalin A (Con A) has been reported to affect plasma membrane-bound enzyme activities in lymphocytes [1,2], fat cells [3,4] and liver [3,5,6]. In particular it has been shown that Con A exerts a hormone-like effect on adenylate cyclase [3], and that it is able to act on plasma membrane-bound enzymes by a mechanism probably related to a modification of cyclic AMP levels [6]. The mitogenicity of Con A [7] has been recently connected with its alleged ability to affect cyclic AMP concentration acting by an insulin-like mechanism on adenylate cyclase [3,8,9], a hypothesis that we were unable to support by closely related experiments [6].

On the basis of the experimental data reviewed above we investigated the possible mechanism by which Con A is able to affect cyclic AMP level; considering both adenylate cyclase and cyclic AMP-phosphodiesterase activity of isolated rat liver plasma membrane. The results obtained suggest that Con A acts, at low concentrations, by inhibiting a plasma membrane-bound high affinity cyclic AMP-phosphodiesterase.

2. Materials and methods

Liver plasma membranes were isolated from male Wistar rats according to Ray [10] as previously reported [11].

Adenylate cyclase was assayed as previously described [12] by measuring cyclic AMP formed according to the method of Brown et al. [13] as previously reported [14].

Cyclic AMP-phosphodiesterase was assayed basically by the two-step procedure of Thompson and Appleman [15] in a medium containing 40 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 80–120 μg membrane protein in a final volume of 0.6 ml [16], unless otherwise stated.

Protein was estimated by the method of Lowry et al. [17], using bovine serum albumin as a standard.

Con A was the twice-crystallized product from Miles-Yeda Ltd., Rehovot, Israel. Cyclic AMP (adenosine 3′,5′ monophosphoric acid) was from Boehringer, Mannheim, Germany. α-D-Methylmannoside was from Sigma, St. Louis, Mo., USA. [8-^{3}H] Adenosine-3′,5′-monophosphoric acid, ammonium salt (27 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks., UK. For the phosphodiesterase assay, freeze-dried 5′-nucleotidase from *Crotalus adamanteus* (BDH Chemicals, Poole, UK) and AG 1-X2, 200–400 mesh, Cl^- form resin (Bio-Rad Laboratories, Richmond, Cal., USA) were employed.

3. Results

The effect of different concentrations of Con A on adenylate cyclase activity of liver plasma membrane has been tested either in basal conditions or in the presence of hormonal activators of the cyclase activity like glucagon and prostaglandin E_1 [12]. The lectin is able to stimulate signficantly adenylate cyclase activity (fig.1) at low concentrations, while concentrations

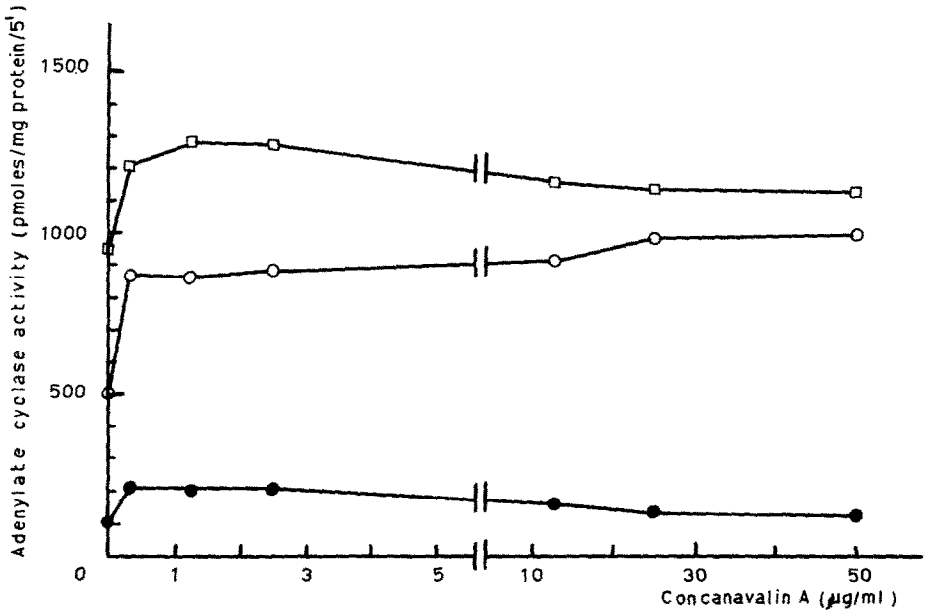

Fig.1. Effect of Concanavalin A on the basal (•————•), prostaglandin E_1 (10 μg/ml)-stimulated (○————○) and glucagon (1×10^{-6} M)-stimulated (▫————▫) adenylate cyclase activity of rat liver plasma membranes. Membranes were preincubated 5 min with or without Concanavalin A. Results are reported as pmoles cyclic AMP/mg protein per 5 min. Points are means of at least two separate experiments carried out in duplicate, the standard error never exceeded 13% of the reported values. Analysis of variance of these data as a randomized-block experiment showed significant ($P<0.05$) differences between the three treatments.

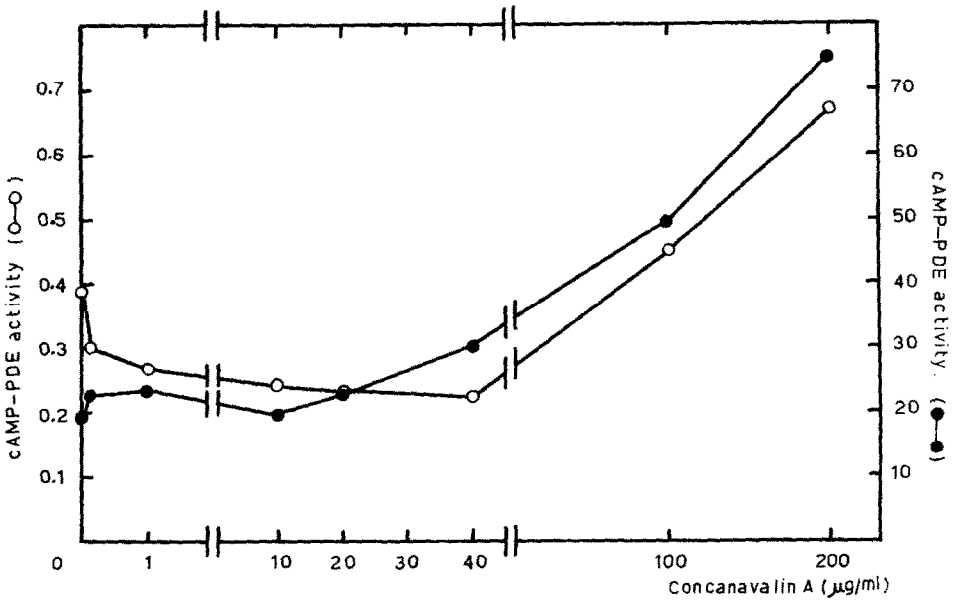

Fig.2

above 5 μg/ml progressively lead to the disappearance of the stimulatory effect, even if this decrease is not statistically significant. More dramatic results are obtained when Con A is tested in the presence of glucagon or prostaglandin E_1: the hormonal stimulation of the adenylate cyclase activity above its basal level is remarkably enhanced by the lectin. In the presence of Con A the effect of prostaglandin E_1 on adenylate cyclase is almost doubled and glucagon effect increases by about 50%, at least up to 3 μg/ml of lectin.

Cyclic AMP-dependent phosphodiesterase activity of liver plasma membrane was investigated, employing two different substrate concentrations, in order to test the low and high affinity component of the enzyme [16,18–20]. Con A added in the range 0.1–40 μg/ml (fig.2) produces a clear and significant inhibitory effect on the high affinity component (tested at 1×10^{-6} M cyclic AMP) while at higher lectin concentrations a paradoxical stimulation, similar to the Con A effect on adipocyte adenylate cyclase [3], occurs. The low affinity component of cyclic AMP-phosphodiesterase (tested at 1×10^{-4} M cyclic AMP) is essentially unaffected by low lectin concentrations, but at high levels (> 20 μg/ml) a stimulatory effect which parallels that seen for the high affinity compo-

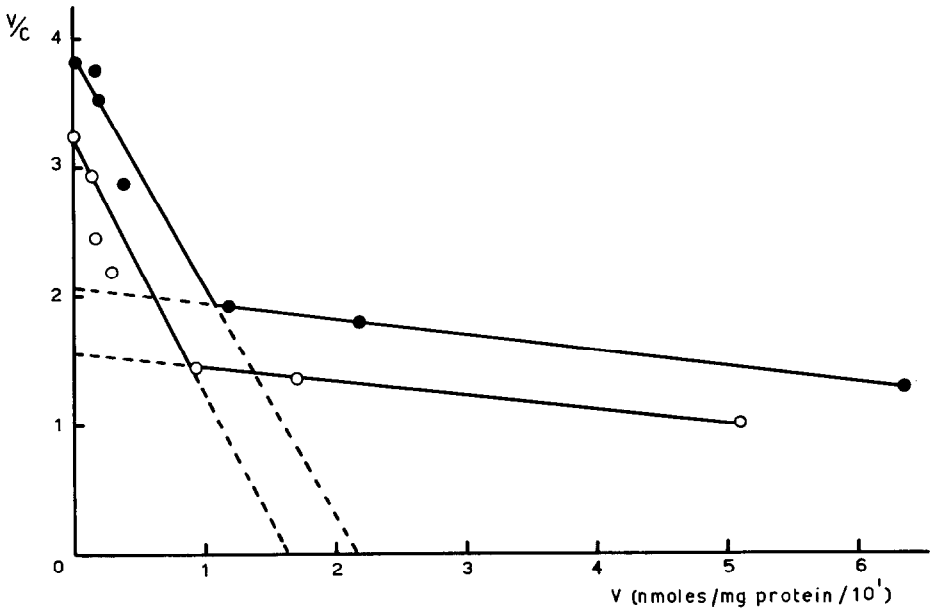

Fig.3. Scatchard-type plot [21] of the effect of Concanavalin A (25 μg/ml) on membrane-bound cyclic AMP-phosphodiesterase. Each point is the mean of triplicate determinations. *C* is given as nmoles of cylic AMP and *V* is expressed as nmoles cyclic AMP hydrolyzed/mg protein per 10 min. Membranes were preincubated 5 min with (○———○) or without (●———●) Concanavalin A before the experiment. The final reaction volume of these experiments was 0.2 ml. Intercepts values were calculated by linear regression. The two intercepts on the *x* axis for the high affinity component are significantly different ($P<0.05$) as assessed by analysis of variance of the regression lines.

Fig.2. Concanavalin A effect on cyclic AMP-dependent phosphodiesterase (cAMP-PDE). Results are reported as nmoles cyclic AMP hydrolyzed/mg protein per 10 min. Substrate concentration was 1×10^{-4} M (●———●) or 1×10^{-6} M (○———○). Plasma membranes were preincubated 30 min at 30°C, with or without the lectin, in the reaction medium before adding the substrate. Each point is the mean of four duplicate experiments carried out on different membrane preparations; the average standard error never exceeded 15% of the reported values. Analysis of variance carried out for the range 0–40 μg/ml of Concanavalin A suggests that the reported effect is statistically significant ($P<0.05$) for cAMP 1×10^{-6} M but not significant for cAMP 1×10^{-4} M.

Table 1
Effects of concanavalin A, α-D-methylmannoside and theophylline on cyclic AMP-phosphodiesterase of liver plasma membranes

	Cyclic AMP 1×10^{-4} M	Cyclic AMP 1×10^{-6} M
Exp. 1		
Control	–	0.32 ± 0.06
Concanavalin A 10 μg/ml	–	0.18 ± 0.02[a]
α-D-Methylmannoside 8 mM	–	0.32 ± 0.06
Concanavalin A 10 μg/ml + α-D-methylmannoside 8 mM	–	0.23 ± 0.03[a,b]
Exp. 2		
Control	26.6 ± 3.8	0.32 ± 0.06
Concanavalin A 10 μg/ml	30.4 ± 4.5	0.21 ± 0.04[a]
Theophylline 5 mM	14.9 ± 2.3[a]	0.33 ± 0.05
Concanavalin A 10 μg/ml + theophylline 5 mM	18.5 ± 2.7[a,b,c]	0.24 ± 0.03[a]

Enzyme activity is reported as nmoles cyclic AMP hydrolyzed ± S.E./mg protein per 10 min. Membranes were preincubated with or without the substances under investigation as reported in fig.2. Results are means of duplicate experiments carried out on five different membrane preparations. Student's t test (n=10) was performed with respect to controls ([a]$P<0.001$); with respect to Concanavalin A ([b]$P<0.001$); with respect to theophylline ([c]$P<0.01$); all other differences being statistically not significant.

nent was observed. A kinetic analysis of the effect of Con A on membrane-bound cyclic AMP-phosphodiesterase (fig.3) shows that the lectin behaves as a non-competitive inhibitor. The following kinetic constants were obtained from the non-linear Scatchard plot extrapolated: for the control K_{M1} and K_{M2} were 2.8 and 41.9 μM respectively; for Con A-treated membranes the values were 2.3 and 47.6 μM. Control V_{max1} and V_{max2} were 2.16 and 17.1, for Con A-treated membranes V_{max1} was 1.5 and V_{max2} 14.8 nmol/mg protein/10 min. Thus the main effect is that the V_{max} of the high affinity component was reduced by Con A. We have also observed that Con A did not affect the activity of cyclic AMP-phosphodiesterase assayed on a soluble fraction obtained after a 30 min centrifugation at 100 000 g of a liver homogenate, when the substrate concentration was varied between 0.04 and 12.5 μM (not shown).

That the Con A inhibition of the high affinity cyclic AMP-phosphodiesterase is dependent on the specific binding of the lectin to plasma membrane-bound saccharide groups is clearly demonstrated by the results reported in table 1, which show that the Con A effect is partially prevented by α-D-methylmannoside, a saccharide which specifically binds to the active site of the lectin [7], but does not itself affect membrane-bound enzyme activities [6]. In addition table 1 shows a clear discrepancy between the effects of Con A and theophylline, a known phosphodiesterase inhibitor [18], on the two components of the cyclic AMP-dependent phosphodiesterase; the low affinity component is inhibited by the methylxanthine but it is insensitive to Con A, whereas essentially the reverse is true for the high affinity component. The latter result gives further support to the differential response of the two phosphodiesterase components to Con A.

4. Discussion

Our results are at variance with published observations [3] concerning a negative modulation of adipocyte plasma membrane adenylate cyclase in the range of low Con A concentrations (up to 30 μg/ml); conversely a paradoxical effect of Con A in the high range of lectin concentrations (40 to 200 μg/ml) has been noticed in our experiments on cyclic AMP-dependent phosphodiesterase. Furthermore the results point to a possible differential separation between low and high affinity cyclic AMP-phosphodiesterase components on the basis of their sensitivity to Con A in the range of low lectin concentrations; such a result, even if with a reversed relationship to the enzyme activity, has already been shown in liver plasma membranes for insulin sensitivity of cyclic AMP-phosphodiesterase [16].

From the data presented so far it is possible to

assume that Con A acts primarily, at low concentrations, as a non-competitive inhibitor of a plasma membrane-bound high affinity cyclic AMP-dependent phosphodiesterase which has been recently described [19,20,22]. Therefore the stimulation exerted by Con A on the basal adenylate cyclase activity as well, as its potentiation of glucagon and prostaglandin E_1 stimulation can probably be ascribed to the phosphodiesterase inhibition; such a hypothesis is well supported by the complete insensitivity of the high affinity phosphodiesterase to theophylline, a substance which is also present during adenylate cyclase assay: this obviously means that the Con A effect is a direct one and it is not a misinterpretation due to the presence of theophylline.

These results which together indicate that Con A acts at the plasma membrane level increasing cyclic AMP concentration rule out, at least for liver, a possible insulin-like effect of the lectin and fit well with previous reports concerned with hormone and Con A sensitivity of membrane-bound enzymes [6,23–25].

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