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EFFECT OF COLCHICINE ON RAT LIVER PLASMA MEMBRANE

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Summary

Colchicine effect has been tested on rat liver plasma membrane-bound enzymes after in vitro or in vivo treatment. It appears that the in vitro treatment does not affect 5′-nucleotidase, Mg^{2+}-ATPase and (Na^++K^+)-ATPase, whereas adenylate cyclase is sensitive to both in vitro and in vivo treatment, the latter condition being also effective for 5′-nucleotidase.

Colchicine is known to affect plasma membrane properties with respect to receptor mobility [1], transport mechanisms [2], enzymatic activities [3—6] and lipid fluidity [7]; whether these effects are mediated through the microtubular system or by a direct action on the membrane itself is still to be established [3].

The effect of colchicine on liver is mainly concerned with secretory processes [8, 9] and, in addition, the presence of colchicine-binding proteins [10] as well as that of a microtubular system [11] have been reported.

Results to be reported herein deal with the in vitro and in vivo effect of colchicine and its inactive analog, lumicolchicine [2], on rat liver plasma membrane-bound enzymes, hormonal response and lipid fluidity.

Male Sprague-Dawley rats (150 g, av. body wt.) were used during this research and were fed ad libitum. For in vivo experiments, animals were injected intraperitoneally with colchicine or lumicolchicine, 1 mg/100 g body wt., dissolved in physiological saline; control animals received an equal volume of saline. Colchicine-treated, as well as control animals, were killed 4 h after injection.

Liver plasma membranes were isolated according to Ray [12] as previ-

ously reported [13]. 5′-Nucleotidase (EC 3.1.3.5), Mg^{2+}-ATPase, (EC 3.6.1.3) and (Na^++K^+)-ATPase (EC 3.6.1.4) were assayed as already reported [14]; adenylate cyclase (EC 4.6.1.1) was assayed as previously reported [15] with the addition of 10 mM GTP when glucagon effect was tested; tissue level of cyclic AMP was established as already reported [16]. Proteins were estimated by the method of Lowry et al. [17] using bovine serum albumin as a standard.

Glucagon was from Sigma (St. Louis, MO, U.S.A.), colchicine was from Merck-Darmstadt (F.R.G.), whereas lumicolchicine was prepared according to Wilson and Friedkin [18]. ATP, AMP and cyclic AMP were from Boehringer-Mannheim (F.R.G.). [8-^{3}H] Adenosine 3′,5′-monophosphoric acid, ammonium salt, was obtained from the Radiochemical Centre, Amersham, Bucks., U.K.

Fluorescence-labeling of plasma membranes was carried out as follows: $2 \cdot 10^{-3}$ M 1,6-diphenyl-1,3,5-hexatriene (Fluka A.G., Buchs, Switzerland) in tetrahydrofuran was diluted 1:1000 prior to use with Tris-buffered saline (0.05 M Tris-HCl, pH 7.5; 0.1 M NaCl) and then mixed in a 1:1 ratio with the plasma membrane suspension in the same medium to give a final protein concentration of approx. 50 μg/ml. This mixture was then incubated for 30 min at 37° C in the presence or absence of the substances under investigation. Fluorescence polarization was measured, as recently reported [19], with an Aminco-Bowman spectrophotofluorometer equipped with two Glan prism polarizers, the excitation was set at 366 nm and the emission at 430 nm. The degree of fluorescence polarization, P, was calculated from the equation:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} = \frac{(I_{\parallel}/I_{\perp}) - 1}{(I_{\parallel}/I_{\perp}) + 1}$$

where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities observed with the analysing polarizer oriented, respectively, parallel and perpendicular to the direction of the polarized excitation beam [20].

5′-Nucleotidase, Mg^{2+}-ATPase and (Na^++K^+)-ATPase activities appear not to be affected by an in vitro treatment with colchicine in a wide range of concentrations, whereas the in vivo treatment significantly reduces 5′-nucleotidase activity leaving both Mg^{2+}-ATPase and (Na^++K^+)-ATPase activities unaltered, (Table I); lumicolchicine, the structurally inactive analog [2] of colchicine was not effective in any case. As far as the in vitro treatment is concerned, our results are at variance as to ATPase activities with previously-published observations [3] regarding the concentration range chosen, but in our case the maximal in vitro concentration was very close to the highest level attainable after the in vivo treatment.

Conversely, plasma membrane-bound adenylate cyclase is markedly sensitive to colchicine treatment, both in vitro and in vivo, even if the in vitro treatment does not show a dose-dependent sensitivity (Table II). Adenylate cyclase of plasma membranes isolated from control injected animals shows a glucagon responsiveness which was nearly abolished by an in vitro treatment with colchicine; on the other hand, the cyclase activity in colchicine-treated animals is significantly reduced but still sensitive to glucagon stimulation ($p < 0.01$). These observations are strengthened by the significant fall of tis-

TABLE I

EFFECT OF AN IN VITRO OR IN VIVO COLCHICINE TREATMENT ON LIVER PLASMA MEMBRANE 5′-NUCLEOTIDASE AND ATPase ACTIVITIES

5′-Nucleotidase activity is reported as μmol P_i hydrolyzed ± S.E. per mg protein per h; ATPase activities are reported as μmol P_i hydrolyzed ± S.E. per mg protein per 5 min. The number of individual membrane preparations tested is reported between brackets; [a] $p < 0.05$ at least, as estimated by Student's *t*-test with respect to controls, all other differences being statistically insignificant. Isolated liver membranes were incubated for 30 min at 37°C with or without colchicine or lumicolchicine at the final concentration indicated; for in vivo treatment see text. In vivo controls were not significantly different from the reported ones.

Treatment	5′-Nucleotidase	Mg^{2+}-ATPase	$(Na^+ + K^+)$-ATPase
In vitro			
control	26.61 ± 1.45 (8)	4.35 ± 0.54 (6)	1.19 ± 0.17 (6)
colchicine $1 \cdot 10^{-7}$ M	28.83 ± 2.34 (3)	4.28 ± 0.47 (6)	1.17 ± 0.24 (6)
colchicine $1 \cdot 10^{-6}$ M	28.16 ± 2.20 (3)	4.44 ± 0.42 (6)	1.12 ± 0.13 (6)
colchicine $1 \cdot 10^{-5}$ M	27.93 ± 1.48 (3)	4.62 ± 0.44 (6)	1.41 ± 0.19 (6)
colchicine $1 \cdot 10^{-4}$ M	28.97 ± 1.66 (4)	4.34 ± 0.57 (6)	1.26 ± 0.22 (6)
lumicolchicine $1 \cdot 10^{-4}$ M	22.90 ± 1.50 (3)	—	—
In vivo			
colchicine	12.00 ± 0.69[a] (6)	4.72 ± 0.38 (6)	1.14 ± 0.08 (6)
lumicolchicine	23.40 ± 1.80 (3)	—	—

sue level of cyclic AMP elicited by the in vivo colchicine treatment (Table II). Lumicolchicine did not affect liver plasma membrane adenylate cyclase, both in vitro (not shown) and after in vivo treatment, as well as its glucagon responsiveness. In addition, 10 mM fluoride-stimulated adenylate cyclase was never affected by both in vitro and in vivo colchicine treatment under the conditions reported so far (not shown). The results concerned with adenylate

TABLE II

COLCHICINE EFFECT ON PLASMA MEMBRANE-BOUND ADENYLATE CYCLASE AND CYCLIC AMP LEVELS OF HEPATIC TISSUE

Adenylate cyclase activity is reported as pmol cyclic AMP ± S.E. per mg protein per 10 min; cyclic AMP concentration of hepatic tissue is given as pmol cyclic AMP ± S.E. per mg wet wt. The number of liver preparations tested is reported between brackets; [a] $p < 0.05$ at least, as estimated by Student's *t*-test when compared to respective controls. For further details see the text and Table I.

Treatment	Adenylate cyclase activity	Tissue level of cyclic AMP
In vitro		
control	35.6 ± 3.2 (6)	—
colchicine $1 \cdot 10^{-7}$ M	38.0 ± 4.6 (3)	—
colchicine $1 \cdot 10^{-6}$ M	37.0 ± 2.0 (3)	—
colchicine $1 \cdot 10^{-5}$ M	19.3 ± 2.2[a] (6)	—
colchicine $1 \cdot 10^{-4}$ M	21.1 ± 4.0[a] (6)	—
In vivo		
control	38.0 ± 4.1 (6)	0.675 ± 0.012 (3)
control + glucagon $1 \cdot 10^{-7}$ M	97.0 ± 7.2[a] (3)	—
control + glucagon $1 \cdot 10^{-7}$ M + colchicine $1 \cdot 10^{-4}$ M added in vitro	44.0 ± 3.5 (3)	—
colchicine	22.2 ± 1.2[a] (6)	0.362 ± 0.010[a] (3)
colchicine + glucagon $1 \cdot 10^{-7}$ M	32.5 ± 1.8 (3)	—
lumicolchicine	32.2 ± 3.0 (4)	0.712 ± 0.037 (3)
lumicolchicine + glucagon $1 \cdot 10^{-7}$ M	93.5 ± 6.9[a] (3)	—

TABLE III

FLUORESCENCE POLARIZATION VALUES ($P \pm$ S.E. AT 37°C) OF LIVER PLASMA MEMBRANES ISOLATED FROM UNTREATED OR COLCHICINE TREATED ANIMALS

Colchicine treatment is described both in the text and in Table I. The number of plasma membrane preparations tested is reported between brackets; differences reported are not statistically significant.

Treatment	P
In vitro	
control	0.254 ± 0.014 (12)
colchicine $1 \cdot 10^{-7}$ M	0.248 ± 0.013 (11)
colchicine $1 \cdot 10^{-6}$ M	0.248 ± 0.054 (12)
colchicine $1 \cdot 10^{-5}$ M	0.245 ± 0.017 (12)
colchicine $1 \cdot 10^{-4}$ M	0.269 ± 0.016 (11)
In vivo	
control	0.242 ± 0.010 (4)
colchicine	0.240 ± 0.011 (4)

cyclase activity after both in vitro and in vivo treatment are comparable to similar experiments carried out in vitro on liver membranes with the mitotic inhibitor, vinblastine [3]. In this context one could recall similar results obtained in Ehrlich cell plasma membrane adenylate cyclase experiments after colchicine treatment [6], whereas different results were obtained in human macrophages concerning both cyclase and cyclic AMP levels [4, 5].

Experiments carried out in order to detect a possible involvement of plasma membrane lipid microviscosity [20] following an in vitro or in vivo treatment with colchicine failed to give any significant indication (Table III), thus confirming comparable observations carried out on a different cellular system [7]; the lack of significant changes of fluorescence polarization values rules out any involvement of membrane lipid fluidity as to the reported effects of colchicine.

If on the one hand, the in vivo effect of colchicine on 5′-nucleotidase and adenylate cyclase activities could suggest the possible involvement of microtubular structures, the clear-cut interaction of the drug in vitro and in vivo with the adenylate cyclase and its hormonal sensitivity is suggestive of a more direct effect on plasma membrane structure, perhaps in connection with a receptor system, which could in some way interplay with the physiological hormonal regulation of the cyclase activity.

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