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Effects of Δ^9-THC on Human Platelet Phospholipids

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KALOFOUTIS, A., J. LEKAKIS AND A. KOUTSELINIS. *Effects of Δ^9-THC on human platelet phospholipids.* PHARMAC. BIOCHEM. BEHAV. 12(5) 697–699, 1980.—The possible change in Platelet lipids after smoking Δ^9-THC was studied in chronic hashish users. The fluctuations of total phospholipid content is related to alterations of individual phospholipids. Changes in phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine are discussed in relation to membrane derangement leading to the increased rate of platelet lysis and aggregation under high doses of the drug.

Platelets Total lipids Phospholipids Δ^9-THC

THE effect of active cannabis constituents on blood platelets is well known. Following drug intake platelet reduction, change of their shape, disturbances of their ADP-induced aggregation and osmotic fragility have been observed [9, 10, 13]. On the other hand active cannabis constituents (e.g. Δ^9-THC) influence the concentration of cell membrane lipids and possibly affect their enzymic systems [8,11].

Since changes in platelet phospholipid concentrations could play a role in the blood coagulation mechanism and consequently in the reduction of platelet count [6,19], we attempted an investigation concerning possible fluctuations of platelet phospholipids in chronic hashish smokers under known experimental conditions [11].

METHOD

In the present study the platelet phospholipid concentration was determined in 20 subjects who were divided into two groups: Group 1 consisted of 10 volunteers, heavy hashish smokers (45–55 years old) who had been using the drug for 20–30 years. Some of them had obvious signs of chronic cannabismus, which means that they were dependent on any kind of hashish preparations (chronic hashish users). Information about the hashish users, the kind and the amount of hashish smoked and the design of the experimental schedule are described in detail elsewhere [11]. Group 2 included 10 healthy individuals (42–53 years old) of low socioeconomic class who consumed no cannabinoids or other drugs, but smoked 30–40 cigarettes or a pipe daily. We chose tobacco smokers as controls in order to have a control group in as similar conditions as possible with those of hashish smokers. In all cases blood samples were drawn before and 30–60 min after smoking, when the highest concentration of the drug in the blood stream is noted [17].

Blood samples were drawn from an antecubital vein, transferred and mixed immediately into siliconized capillary tubes with E.D.T.A. 1.5% (pH 7.26) 4:1 v/v. Samples were centrifuged at 90 g (MSE, Mistral 6L) for 10 min at 4°C. The supernatant platelet rich plasma (the upper 2/3 of the whole plasma volume) was obtained by gentle circular motion with a pasteur pipette and centrifuged at 4,340 g (Sorvall-RC2B) for 15 min. The pellet was washed twice with 0.9% NaCl solution and centrifuged at 4,340 g for 15 min again to obtain better purification.

The platelet pellet was then analyzed for total lipids according to the method of Folch *et al.* [4]. The separation of total lipids into neutral lipid, glycolipid and phospholipid fractions was achieved using a silicic acid column special for lipid chromatography as described in a previous work [7]. The phospholipid classes by two dimensional thin-layer chromatography [12] on precoated 10×10 cm Silica gel G plates (Merck, Darmstadt, GFR). The individual phospholipid spots were visualized as follows: The lipid containing ninhydrin-reacting residue by spraying with a ninhydrin solution (ninhydrin 1 g/l and pyridine 3:1 v/v) and heating to 80°C for 10–12 min. Each phospholipid class was analyzed in duplicate by chromatography using commerical phospholipids as standards (Pierce Chem. Co., Rockford, IL, U.S.A.). The lipid phosphorus was estimated following Bartlett's method [2]. The statistical analysis was carried out by Student's *t*-test.

RESULTS

The results of this study are presented in Table 1 and 2. Table 1 shows that no statistically significant differences exist in total lipid and phospholipid concentrations before and after smoking tobacco. Likewise no differences in individual phospholipid concentrations were noticed. From Table 2 it can be seen that the total lipid concentration did not vary before and after the intake of the drug, while total phospholipids changed significantly after smoking hashish ($p<0.01$).

Further analysis of the individual phospholipid fractions revealed the following interesting fluctuations: (a) A significant decrease of phosphatidylcholine ($p<0.01$) and phos-

TABLE 1

PERCENTAGE CONCENTRATION OF PLATELET PHOSPHILIPIDS IN HEALTHY CONTROLS (VALUES ARE EXPRESSED AS MEAN ± SD)

Phospholipid class	Controls (n=10)		
	Before*	After*	p
Total lipids	0.79 ± 0.21 mg/10^9	0.76 ± 0.23 mg/10^9	N.S.
Total phospholipids	0.46 ± 0.16 mg/10^9	0.43 ± 0.17 mg/10^9	N.S.
LPC	1.64 ± 0.39	1.69 ± 0.43	N.S.
PC	38.26 ± 3.19	38.16 ± 3.24	N.S.
SPH	17.04 ± 2.10	17.29 ± 2.16	N.S.
PE	27.49 ± 2.92	27.64 ± 2.79	N.S.
PI	9.16 ± 1.06	8.62 ± 0.87	N.S.
PS	5.54 ± 0.73	5.73 ± 0.84	N.S.
DPG	0.78 ± 0.34	0.79 ± 0.29	N.S.

*Smoking tobacco.

Platelets=mg/10^9 cells. N.S.=not significant. LPC=lysophosphatidylcholine, PC=phosphatidylcholine, SPH=sphingomyelin, PE=phosphatidylethanolamine, DPG=diphosphatidylglycerol. Phospholipid fractions were separated by two dimensional thin-layer chromatography as follows: (a) the first dimension with a $CHCl_3$: CH_3OH : NH_4OH : H_2O 180 : 105 : 7.5 : 7.5 v/v solution; (b) the second dimension with a $CHCl_3$: CH_3OH : NH_4OH : H_2O 120 : 160 : 5 : 5 v/v solution. The phosopholipid components were visualized by spraying the plates with 50% H_2SO_4 and heating at 160°C for 15 min and with 0.1o% ninhydrin-pyridine, 3:1 v/v and heating at 80°C for 10 min.

TABLE 2

PERCENTAGE CONCENTRATION OF PLATELET PHOSPHOLIPIDS IN HASHISH USERS

Phospholipid class	Hashish users (n=10)		
	Before*	After*	p
Total lipids	0.77 ± 0.21 mg/10^9	0.74 ± 0.20 mg/10^9	N.S.
Total phospholipids	0.48 ± 0.15 mg/10^9	0.38 ± 0.14 mg/10^9	0.01
LPC	1.66 ± 0.49	1.92 ± 0.36	N.S.
PC	40.86 ± 3.41	37.40 ± 3.23	0.01
SPH	16.19 ± 2.08	17.55 ± 2.16	N.S.
PE	25.26 ± 2.96	28.32 ± 3.04	0.05
PI	9.55 ± 0.92	7.64 ± 0.89	0.0005
PS	5.53 ± 0.91	6.11 ± 0.84	N.S.
DPG	0.95 ± 0.36	1.04 ± 0.39	N.S.

*Smoking hashish.

Designations as in Table 1.

phatidyllinositol ($p<0.0005$) after smoking hashish. (b) A significant increase in phosphatidylethanolamine ($p<0.05$) after intaking the drug.

Finally comparing Tables 1 and 2 no statistically significant differences between controls and hashish smokers before using the drug were found in the values of total lipids, total phospholipids, and individual phospholipids.

DISCUSSION

It is well known that cannabinoids have a broad spectrum of action on platelet function and structure, which has been correlated to the psychomimetic potency of the drug [14,15]. The reduction in platelet count resulting from the action of cannabinoids could be explained either by the lysis of erythrocytes, release of ADP and increased aggregation [13] or by the direct influence of THC constituents on platelets [21]. The exact mechanism by which THC constituents directly affects the platelets remains unexplained.

Recent studies [1, 8, 11] have demonstrated that administration of THC constituents and especially Δ^9-THC induces membrane lipid and particularly phospholipid changes in

leucocytes, lympocytes and erythrocytes. We emphasize the activity of Δ^9-THC since it has been established that it is the most active and potent constituent of hashish and it is found in the highest concentration [11].

The marked reduction observed in the platelet total phospholipid concentration could be explained as a direct effect of Δ^9-THC on membrane phospholipids, since it is well known that cannabinoids have a lipophilic nature and probably change the membrane functional processes [1, 3, 22]. This reduction in total phospholipid content is followed by a concomitant change of some individual phospholipid fractions. Since it is well known that phosphatidylcholine is required for the activation of some membrane enzymic systems [5,16] and at least high doses of cannabinoids are suspected to have a disordering effect and probably cause a membrane rupture [1,22] we believe that the observed reduction of this phospholipid after smoking hashish could be attributed to a direct effect of Δ^9-THC on platelet membranes, leading to a perturbation of the phospholipid related enzymic mechanism.

Another important finding of the present work is the reduced concentration of phosphatidylinositol after intaking the drug. It is well established that phosphatidylinositol is associated with the function of some membrane surface bound enzymes and plays a fundamental role in ion transport through cellular membranes. The abrupt reduction of this phospholipid could be related to the function of receptor mechanisms occurring on the membrane and possibly alters the transduction of the signal produced by phosphatidylinositol connected enzymic system fluctuations (e.g. adenyl-cyclase) [20].

Finally the increase in concentration of phosphatidylethanolamine after the drug intake observed in the present study could be of interest. This phospholipid has been implicated in some changes in blood functions, such as blood coagulation [18]. On the other hand it appears to be related to the transport of some fatty acids that are involved in the blood viscosity regulatory mechanism. It is therefore possible to relate the present increase of this phospholipid with the changes in blood coagulability after the drug intake [6]. This however requires further research.

The results obtained in this study indicate a possible structural reorganization of platelet membranes, a conclusion supported by Levy *et al.* [14] who proposed that high doses of cannabinoid constituents cause lysis of part of the platelet population, releasing endogenous inducers and thus favoring platelet aggregation.

The conclusions drawn from this study could open the way for further investigation in this field since the phospholipid changes observed in this work could be taken into account to explain platelet lysis and aggregation under high doses of active hashish constituents.

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