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# Changes Induced by Hashish Constituents on Human Erythrocyte Phospholipids

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KALOFOUTIS, A. AND A. KOUTSELINIS. *Changes induced by hashish constituents on human erythrocyte phospholipids*. PHARMAC. BIOCHEM. BEHAV. 11(4) 383–385, 1979.—The possible effect of $\Delta^9$-THC on human erythrocyte phospholipids of chronic hashish users was studied. Changes in individual phospholipids concentrations were observed in heavy hashish users after smoking the drug. Results are discussed in relation to the possible alterations of enzymatic mechanisms which require the presence of essential for their activation phospholipids.

$\Delta^9$-Tetrahydrocannabinol Erythrocytes Total lipids Phospholipids

IT IS well known that cannabinoids have a liphophilic nature and very high membrane/aqueous solution partition coefficient [6,21]. Furthermore they could get inserted into the lipid bilayer and possibly cause a membrane disturbance in the arrangement of phospholipids regions, leading to changes in membrane permeability and inhibition of the membrane function [2,13].

On the other hand, the psychomimetic components of hashish (e.g., THC) influence various membrane systems and moreover are also effective in modifying the erythrocyte membrane; particularly by stabilizing against osmotic lysis [1,19] inhibiting glucose efflux, $Na^+ + K^+$-ATPase and Acyl-transferase activity [2, 9, 20].

All the above mentioned observations lead us to the suggestion that hashish constitutents can exert a remarkable variation on the structural and functional organization of membranes.

The present study was undertaken in order to examine the possibility of erythrocyte lipid changes in chronic hashish users under known experimental conditions [14]

## METHOD

Ten volunteers (age 45 to 55 years) heavy hashish smokers as well as ten healthy subjects (age 42 to 53 years) were used in these experiments. All of the heavy hashish smokers had been using hashish for 20–30 years. Some of them had obvious signs of chronic cannabismus. According to the information given by the subjects they had smoked every kind of hashish product (pure resin, marihuana, etc.) that could possibly be found, using always a nargile pipe. Some of them reported to have smoked, in certain cases, up to 100 g of crude hashish at a time. For each hashish users one control subject was tested at the time. The control group was a low socioeconomic class who consumed no cannabinoids or any other drug, but who smoked 30–40 cigarettes a day or a pipe.

In our experiments pure hashish resin was used which was extracted from the flowering tops of female cannabis plants. It should be taken into consideration that the resin used was first analysed by chromatographic methods, the concentration of active constituents and especially of $\Delta^9$-THC was therefore accurately known (3.6%).

Each of the hashish users were allowed to smoke 20 g of pure resin by nargile pipe for a period not exceeding 15 min. At the same time, and under the same conditions, each of the control subjects smoked about 20 g of pure tobacco by pipe.

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 [18].

Blood samples were drawn from an antecubital vein and transferred immediately into siliconized capillary tubes. After the whole blood leukocyte and platelets separation the erythrocytes residues were mixed 9:1 v/v with 1.5% EDTA solution in 0.9% NaCl and centrifuged at 13.000 rev/min for 30 min at 4°C (Sorvall RC—2B).

The erythrocyte pellet was then analyzed for total lipids according to the method of Folch *et al.* [7]. The separation of total lipids into neutral lipid, glycolipid and phospholipid fractions was achieved using a silicic acid column Special for lipid chromatography, Lab. Richmond, CA, USA. The following solutions were used to elute the lipid classes: Neutral lipids (12 ml of $CHCl_3$), Glycolipids (20 ml of a mixture 9:1 v/v acetone/MeOH) and Phospholipids/12 ml of a mixture $CHCl_3$/MeOH 2:1 v/v [12]. The phospholipid fraction was further analyzed into individual phospholipid classes following two dimensional thin layer chromatography [15] on precoated 10×10 cm Silica gel G plates (Merck, Darmstadt, G.F.R.). The individual phospholipid spots were visualized as follows: (a) The major phospholipid components by spraying the chromatogram with $H_2SO_4/H_2O$ 1:1 v/v and heating to 160°C for 15 min, and (b) the lipid containing ninhydrin-reacting residue by spraying with a ninhydrin solution (ninhydrin 1 g/L and pyridin 3:1 v/v) and heating to 80°C for 10–12 min. Each phospholipid class was analysed in duplicate by chromatography using commercial phos-

TABLE 1
ERYTHROCYTES PHOSPHOLIPID CONCENTRATION IN HEAVY HASHISH USERS AND CONTROLS

| | Controls (n=10) | | | Hashish Users (n=10) | | |
|---|---|---|---|---|---|---|
| | Before | P | After | Before | P | After |
| Total lipids* | 0.45 ± 0.09 | N.S. | 0.43 ± 0.08 | 0.46 ± 0.09 | N.S. | 0.44 ± 0.07 |
| Phospholipids† | 0.33 ± 0.06 | N.S. | 0.34 ± 0.06 | 0.35 ± 0.07 | 0.01 | 0.28 ± 0.04 |
| Lysophosphatidylcholine | 1.36 ± 0.39 | N.S. | 1.40 ± 0.46 | 1.56 ± 0.32 | 0.005 | 2.42 ± 0.74 |
| Phosphatidylcholine | 31.63 ± 2.78 | N.S. | 32.02 ± 2.84 | 33.68 ± 3.32 | 0.05 | 31.20 ± 2.59 |
| Sphingomyelin | 21.04 ± 1.91 | N.S. | 20.68 ± 1.82 | 19.15 ± 1.86 | N.S. | 20.32 ± 1.96 |
| Phosphatidylethanolamine | 28.64 ± 3.04 | N.S. | 28.86 ± 2.95 | 28.50 ± 2.77 | N.S. | 30.29 ± 2.69 |
| Phosphatidylserine | 12.19 ± 1.26 | N.S. | 11.97 ± 1.29 | 12.10 ± 1.14 | 0.01 | 11.06 ± 1.10 |
| Phosphatidylinositol | 3.62 ± 0.64 | N.S. | 3.47 ± 0.57 | 3.59 ± 0.61 | 0.05 | 3.08 ± 0.64 |
| Diphosphatidylglycerol | 0.41 ± 0.11 | N.S. | 0.38 ± 0.09 | 0.45 ± 0.12 | N.S. | 0.50 ± 0.10 |
| Phosphatidic Acid | 1.10 ± 0.26 | N.S. | 1.18 ± 0.22 | 0.99 ± 0.20 | N.S. | 1.11 ± 0.27 |

*Erythrocytes=mg/$10^9$ cells. N.S.=not significant
†Individual phospholipids analytes % mean ± SD

pholipids as standards (Pierce Chemical Co., Rockford, IL, USA). The lipid phosphorous was estimated following Bartlett's method [4].

## RESULTS

As shown in Table 1 the concentration of total erythrocyte phospholipids in heavy hashish users is decreased significantly ($p<0.01$), while no significant change was found in the control group both in the total lipid and phospholipid content.

Regarding the individual phospholipid classes the findings are summarized as follows:

(a) We found no statistical changes of any phospholipid class in the control group before and after smoking an equivalent amount of tobacco.

(b) We found no significant difference between the control group (before and after smoking) and the drug users before smoking hashish.

(c) We observed a decreased concentration of phosphatidylcholine ($p<0.05$), phosphatidylinositol ($p<0.05$) and phosphatidylserine ($p<0.01$) in the hashish users smoking the drug.

(d) We observed an increased concentration of lysophosphatidylcholine ($p<0.005$) in users after smoking hashish.

## DISCUSSION

The results obtained in the present study reflect a biochemical action of hashish components and especially $\Delta^9$-THC on erythrocyte phospholipid concentrations in heavy hashish smokers.

The presence of some phospholipid fluctuations in erythrocytes of hashish users could be related to a perturbation in the structure and function of the erythrocyte membrane. Indeed in this paper we observed a decreased concentration in the total phospholipid content. This finding could be reflected by a change in the individual phospholipid of the erythrocyte membrane. Since it is well known that cannabinoids cause a disturbance in the permeability of the lipid bilayer membrane, damage the mitochondria, and affect function of the lysosomes [3, 5, 10, 16] it is evident that our observation suggests a possible reorganization in membrane lipids induced by chronic drug use.

Concerning the individual erythrocyte phospholipid classes we found some interesting fluctuations in heavy hashish smokers. Regarding phosphatidylcholine, probably the most important structural and functional phospholipid, its decreased concentration in heavy hashish users could be explained by the interaction with THC constituents in a way leading to a functional alteration of some enzymatic membrane systems. This reduction plays an important role as this phospholipid is required for the activation of membrane bound enzymes associated to functional cell processes (Acyl-transferase, etc.) [9,17]. The reduced concentration of phosphatidylcholine should also be connected to the increased concentration of lysophosphatidylcholine, since the existence of a biosynthetic controlling mechanism has been well established [22,23].

Another finding demanding much attention is the reduced concentration of phosphatidylserine and phosphatidylinositol in heavy hashish smokers. It has been established that both phospholipids are strongly associated with a number of membrane functional processes and are required for the activation of some membrane enzymes, e.g., $Na^+ + K^+$-dependent ATRase, Adenylcyclase [2, 8, 24]. It is therefore clear that the reduced concentrations of phosphatidylinositol and phosphatidylserine may be related to the increased concentration of these two phospholipids in plasma [14] and appears to be caused by an interaction with hashish constituents and especially $\Delta^9$-THC. This explanation comes forward to support the findings of other investigators [8] who suggest an inhibition of $Na^+ + K^+$-ATPase activity by the hashish products and which is connected with the function of these two phospholipids [11,13]. It thus seems that the reduction of these components under high concentration of cannabinoids (e.g., $\Delta^9$-THC) probably causes a permanent malfunction of $Na^+ + K^+$-ATPase and other enzymatic membrane systems.

All observations reported in this work point to a damage of the arrangement in the erythrocyte lipid membrane in heavy hashish smokers. This provides a possible explanation—at least in high concentration of $\Delta^9$-THC—for

lysis of erythrocytes membrane, which in turn is incriminated for the platelet reduction exerted by cannabinoids.

The conclusions of our study may be useful for further research in this field concerning the elucidation of mechanisms by which hashish components affect the lipid profile of cell membranes.

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