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Mode of action of hashish compounds in reducing blood platelet count

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Intravenous injection of crude marihuana causes a marked reduction in platelet count [1–3]. Henderson and Pugsley [2] suggested that the particulate matter in the injected marihuana evoked a diffuse intravascular clotting, leading to thrombocytopenia and pulmonary infarction. King *et al.* [3] were unable to substantiate this conclusion. They observed that the addition of marihuana broth to human blood *in vitro* resulted in a profound drop in platelet count [4], implying a direct effect on platelets. We have re-examined this effect, using two defined hashish components, cannabidiol (CBD) and Δ^1tetrahydrocannabinol (THC). Our observations led to the conclusion that the marked reduction in blood platelet count by hashish components is due to an indirect effect, related to lysis of erythrocytes.

Blood was drawn from healthy donors who had received no medication at least during the preceding week. Eighteen-gauged needles were used and the blood was collected into plastic tubes containing heparin (4 i.u./ml final conc). All experiments were conducted within 2 hr after blood collection. Aliquots (5 μl) of cannabinoid stock solutions in 70% ethanol were added with stirring (1000 rev/min, 1 min) to 2 ml blood at 37°, to give a final concentration as desired. A separate control for ethanol itself was included in each case. The blood samples were then kept unstirred at 25° for 30 min, to allow the separation of platelets from other formed bodies by gravitation, and the platelets were counted by a Coulter counter, model Fn. In order to control the ratio of cannabinoid added to platelet count, experiments were limited to blood samples in which platelet count averaged 250–280 per nl.

Table 1 shows that both THC and CBD cause a marked, dose-dependent, decrease in platelet count, THC being more effective particularly at the higher concentrations. All reductions in platelet count are statistically significant, based on *t*-test. A small, but distinct ($p < 0.05$) decrease in platelet count was caused by ethanol itself.

Platelet rich plasma was prepared by centrifuging the whole blood at 120 *g* for 10 min and then recentrifuging the supernatant to remove residual erythrocytes. When platelet rich plasma was incubated for 30 min with either CBD or THC, at concentrations of up to 3×10^{-5} M, no decline in platelet count was evident (Table 2).

It is inferred that the erythrocytes are required for the marked effect of the cannabinoids on platelet count. Observations by phase microscopy taken simultaneously during cell counting revealed clearly the presence of erythrocyte ghosts in blood samples treated by either THC or CBD. Ghosts were rarely observed in ethanol-treated or control samples. To estimate quantitatively the extent of hemolysis, blood samples were treated with THC as described above, except that following the 30 min incubation period, the suspensions were centrifuged at 2000 *g* for 5 min. The supernatants were analyzed spectroscopically at 540 nm. Aliquots (20 μl) of whole blood, diluted with 3 ml H_2O were also sampled to compute the total hemoglobin content. Incubation with 3×10^{-5} M THC resulted in 0·5% hemolysis.

ADP released from the lyzed erythrocytes [5] may induce platelet aggregation, and thus also cause an apparent decline in platelet count. In such a case it is anticipated that the decline in platelet count be prevented by removal of ADP released from the red cells. Removal of ADP can be accomplished either by hydrolysis with apyrase or by phosphorylation with pyruvate kinase and phosphoenolpyruvate. Table 3 shows that addition of apyrase to whole

Table 1. Platelet count of cannabinoid-treated blood

		Platelet count* (per nl)	Per cent of control
Control		254 ± 10	100
Ethanol	4×10^{-5} M	220 ± 4	89
THC	10^{-7} M	178 ± 6	70
	10^{-6} M	145 ± 7	57
	10^{-5} M	129 ± 5	51
	3×10^{-5} M	116 ± 3	46
CBD	10^{-7} M	198 ± 10	78
	10^{-6} M	178 ± 8	69
	10^{-5} M	157 ± 5	62
	3×10^{-5} M	154 ± 2	61

* Average values from 5 experiments run in triplicates ± S.E.

Table 2. Platelet count of platelet rich plasma treated with cannabinoids

		Platelet count* (per nl)
Control		276 ± 2
Ethanol	4×10^{-5} M	275 ± 3
THC	3×10^{-5} M	278 ± 3
CBD	3×10^{-5} M	276 ± 3

* Average values from 2 experiments run in triplicate.

Table 3. Platelet count of cannabinoids-treated blood in presence of apyrase*

		Platelet count† (per nl)	Per cent of control
Control		249 ± 2	100
Ethanol	4×10^{-5} M	251 ± 2	101
THC	3×10^{-5} M	236 ± 6‡	95
CBD	3×10^{-5} M	252 ± 5	101

* Grade I, Sigma Chemical Co., containing ATPase and ADPase activities; 0·25 units ADPase/ml added (one unit of activity will liberate 1 μmole of inorganic phosphate/min at 30°).

† Average values from 3 experiments run in triplicate.

‡ Not significantly different from control, $p > 0.05$ (*t*-test).

* One unit will convert 1·0 μmole of phosphoenolpyruvate to pyruvate per min at pH 7·6 at 37°.

blood, just prior to the cannabinoids, does indeed prevent the reduction of platelet count. Similar results were obtained when the blood was fortified with the following compounds (volume added and final activity or concentration in blood, respectively): pyruvate kinase (10 μl, 5 units*/ml), phosphoenolpyruvate (10 μl, 0·28 mM) and $MgCl_2$ (5 μl, 0·8 mM). This phosphorylating system is known to prevent ADP-mediated platelet aggregation by effectively removing ADP from the medium [6].

It is concluded that the hashish components added to blood cause the release of erythrocyte ADP, which, in turn, induces platelet aggregation and thus, eventually, the well documented reduction of platelet count is apparent. The involvement of erythrocytes in this chain of events appears likely in view of their overwhelming abundance in blood, relative to thrombocytes. Over 90% of the adenine nucleotides of whole blood is in the red cells and indeed, ADP released from erythrocytes by hemolysis is considered to play a physiological role in the initial stages of hemostasis [7]. It is of interest that the concentrations of 10^{-6}–10^{-5} M THC, which are effective in reducing the platelet count in blood, are also effective in modifying the erythrocyte membrane [8, 9] and correlate with the doses leading to psychomimetic reactions in hashish smokers [10, 11].

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Department of Biology and Research & Development Authority, Ben-Gurion University of the Negev, Beer-Sheva, Israel

RACHEL LEVY
AVINOAM LIVNE

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Inability of methadone to prevent the depletion of brain 5-hydroxyindoles by *p*-chloroamphetamine

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Ciofalo[1] has reported that methadone inhibits the uptake of serotonin into brain synaptosomes *in vitro*. This finding raises the question of whether inhibition of serotonin reuptake by methadone occurs *in vivo*. One means of evaluating uptake into serotonin neurons *in vivo* involves the use of depleting agents that require active transport into the serotonin neuron via the uptake pump on the neuronal membrane. *p*-Chloroamphetamine (PCA) is such an agent. The depletion of brain serotonin by *p*-chloroamphetamine is prevented by inhibition of (its) uptake into serotonin neurons [2–4]. We have, therefore, determined whether methadone affects the depletion of brain serotonin by *p*-chloroamphetamine.

Male albino rats (150 g) or mice (20 g) were obtained from a local breeder. Methadone hydrochloride in the racemic or stereoisomeric form was synthesized at Eli Lilly & Co., and *p*-chloroamphetamine hydrochloride was purchased from the Regis Chemical Co. After they were treated, the animals were killed by decapitation, and whole brains were rapidly removed and frozen on dry ice. The brains were stored in a freezer prior to analysis of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) levels by a fluorometric method involving condensation with *o*-phthalaldehyde [5, 6].

Table 1 shows that the depletion of brain serotonin and 5-HIAA by *p*-chloroamphetamine was not altered significantly by *l*-methadone at doses of 1–10 mg/kg, i.p. Higher doses could not be used because of toxicity; two rats treated with *l*-methadone alone at the 10 mg/kg dose died, and one rat treated with *l*-methadone at that dose plus *p*-chloroamphetamine died. In contrast, Lilly 110140, a known inhibitor of serotonin uptake *in vitro* and *in vivo* [7, 8], completely antagonizes the depletion of serotonin by *p*-chloroamphetamine [9]. Since continual reuptake of *p*-chloroamphetamine is apparently required for

Table 1. Failure of *l*-methadone pretreatment to prevent the depletion of rat brain 5-hydroxyindoles by *p*-chloroamphetamine*

Treatment group	N	Brain 5-hydroxyindoles (μg/g) Serotonin	5-HIAA
Control	5	0·49 ± 0·03	0·40 ± 0·01
PCA	5	0·27 ± 0·03†	0·30 ± 0·02†
PCA + *l*-methadone (1)	5	0·23 ± 0·01†	0·29 ± 0·01†
PCA + *l*-methadone (3)	5	0·25 ± 0·01†	0·27 ± 0·004†
PCA + *l*-methadone (10)	3	0·33 ± 0·05‡	0·32 ± 0·03‡
l-Methadone (10)	4	0·46 ± 0·02	0·41 ± 0·02

* PCA (10 mg/kg, i.p.) was injected 10 min after methadone and 4 hr before the rats were killed. *l*-Methadone was injected (i.p., mg/kg) at the doses indicated in parentheses. Mean values ± standard errors are shown.

† $P < 0·001$, different from control.

‡ $P < 0·025$, different from control.