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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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.

Table 2. 5-HIAA levels in mouse brain after methadone treatment*

Treatment group	N	5-HIAA (μg/g)
Experiment 1		
Control	5	0·42 ± 0·02
dl-Methadone (5 mg/kg)	3	0·50 ± 0·01 (NS)
dl-Methadone (10 mg/kg)	3	0·48 ± 0·06 (NS)
dl-Methadone (15 mg/kg)	3	0·46 ± 0·005 (NS)
d-Methadone (5 mg/kg)	3	0·46 ± 0·005 (NS)
d-Methadone (10 mg/kg)	3	0·48 ± 0·02 (NS)
d-Methadone (15 mg/kg)	3	0·50 ± 0·02 (NS)
l-Methadone (5 mg/kg)	3	0·46 ± 0·005 (NS)
l-Methadone (10 mg/kg)	3	0·47 ± 0·02 (NS)
l-Methadone (15 mg/kg)	3	0·44 ± 0·02 (NS)
Experiment 2		
Control	5	0·39 ± 0·03
Probenecid (200 gm/kg)	5	0·72 ± 0·04 ($P < 0·001$)
Probenecid (200 mg/kg) + *dl*-methadone (15 mg/kg)	5	0·64 ± 0·02 ($P < 0·001$)†

* Mice were killed 2 hr after the i.p. injection of methadone. In Experiment 2, probenecid was injected i.p. 15 min after methadone. Mean values ± standard errors are shown. NS = not significant.

† Not significantly different from group receiving probenecid alone.

maintenance of serotonin depletion, the ability of a drug to block *p*-chloroamphetamine reflects inhibition of uptake into serotonin neurons throughout the 4-hr period after *p*-chloroamphetamine injection. Misra and Mule [10] have shown that the initial half-life of the *l*-isomer of methadone in rat brain is about 2·4 hr, with appreciable drug levels still present after 6 hr; thus, the failure of methadone to block *p*-chloroamphetamine could probably not be attributed to insufficient duration of action. Carlsson and Lindqvist [11] earlier observed that methadone at the highest dose that could be used without toxicity was only a very weak antagonist of serotonin depletion by 4-methyl-α-ethyl-*m*-tyramine in mice.

Also of interest is the failure of methadone alone to change 5-HIAA levels (Table 1). Lilly 110140 reduces 5-HIAA levels by decreasing serotonin turnover [12]. This action (reduction of serotonin turnover and lowering of 5-HIAA levels) appears to be common to all known inhibitors of serotonin reuptake, e.g. chlorimipramine and imipramine [13–22]. Thus, the failure of methadone to lower 5-HIAA levels is further evidence that it does not inhibit serotonin reuptake at the doses reported in Table 1 and is in agreement with other reports that methadone does not change 5-HIAA levels [23, 24] or alter serotonin turnover [24] in rat brain.

Ciofalo [1] cited the report by Bowers and Kleber [25] that methadone elevated brain 5-HIAA levels in mice. He suggested that the increased 5-HIAA levels could occur as a consequence of the block of serotonin reuptake, but in fact an increase in 5-HIAA is just opposite to what actually happens when serotonin reuptake is inhibited (see above paragraph). To see if the lack of change in 5-HIAA levels after methadone injection into rats might be attributed to a species difference, we studied methadone in mice at the same doses, routes of injection, and times that Bowers and Kleber used in acute experiments. Table 2 shows that neither isomer of methadone, nor *dl*-methadone, had any effect on 5-HIAA levels in mouse brain. Likewise, *dl*-methadone did not affect the accumulation of 5-HIAA caused by probenecid administration. Thus, our data in mice not only suggest that methadone does not inhibit serotonin reuptake but also fail to confirm the report of Bowers and Kleber that acute treatment with methadone increases 5-HIAA levels or turnover. The failure of methadone to alter 5-HIAA accumulation after probenecid in mice agrees with the data of Goodlet and Sugrue [24] in rats.

From these findings we infer that methadone does not inhibit uptake into serotonin neurons at doses known to be effective in causing analgesia [26] and, therefore, suggest that inhibition of serotonin reuptake does not play a part in the pharmacologic effects of methadone.

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