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Biochemical Pharmacology 61 (2001) 1357–1367

Biochemical Pharmacology

Cannabinoid-induced alterations in brain disposition of drugs of abuse

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Received 27 July 2000; accepted 4 October 2000

Abstract

Marijuana contains a complex mixture of compounds including tetrahydrocannabinol (THC), the major psychoactive constituent, and cannabidiol (CBD), a nonpsychoactive constituent. We have shown previously that CBD pretreatment of mice increases brain levels of THC and have now further characterized this effect and determined whether the brain pharmacokinetics of other drugs are also affected. CBD pretreatment of mice (30–60 min) increased brain levels of THC nearly 3-fold, whereas CBD co-administration did not. Because marijuana is often consumed with other drugs, the influence of cannabinoids on the brain levels of several other drugs of abuse was also determined. CBD pretreatment of mice increased brain levels (2- to 4-fold) of subsequently administered cocaine as well as phencyclidine (PCP). Although CBD pretreatment increased blood and brain levels of cocaine comparably, blood levels of PCP were only modestly elevated (up to 50%). Behavioral tests indicated that the CBD-mediated increases in the brain levels of THC, cocaine, and PCP correlated with increased pharmacological responses. Pretreatment with THC instead of CBD could similarly increase brain levels of cocaine, PCP, and CBD, although with a lower potency than CBD. On the other hand, pretreatment of mice with CBD had no effect on the brain levels of several other drugs of abuse including morphine, methadone, or methylenedioxyphenyl-methamphetamine. These findings demonstrate that cannabinoids can increase the brain concentrations and pharmacological actions of several other drugs of abuse, thereby providing a biochemical basis for the common practice of using marijuana concurrently with such drugs.

Keywords: Cannabidiol; Tetrahydrocannabinol; Pharmacokinetics; Marijuana; Drugs of abuse; Plasma protein binding

1. Introduction

THC is the principal psychoactive constituent of marijuana [1] and possesses several pharmacological properties that may be therapeutically useful [2]. Marijuana is consumed by millions of Americans and very often is ingested with other drugs of abuse. Marijuana contains a complex mixture of dozens of different cannabinoids along with many other less characterized phytochemicals. CBD is another major cannabinoid constituent found in marijuana, and although it is not psychoactive [3,4], it can inhibit the hepatic microsomal metabolism of THC as well as that of many other compounds in mice [5–7]. CBD has also been shown to alter the effects of THC in laboratory animals, as well as in human subjects [8–11]. CBD treatment attenuates THC-induced psychological disturbances such as feelings of anxiety and panic after administration to human subjects [9–11]. Since CBD is not psychoactive and binds extremely weakly to cannabinoid receptors in the brain [4], CBD-mediated modulation of THC activity is probably not pharmacodynamic in nature but may be due to its effects on THC metabolism and/or disposition. In fact, we have shown previously [12] that CBD pretreatment increases brain levels of THC and, to an even greater extent, that of its primary oxidative metabolites. These increases in THC and its metabolites in the brain are much larger than would be predicted from their blood levels, which were only modestly affected by CBD pretreatment. Cannabinoid interactions that modulate brain levels of THC and its metabolites may contribute to the preference that individuals (using marijuana as a therapeutic agent) show for marijuana over THC alone. In addition, if cannabinoids are also found to increase brain levels of other abused drugs, it would provide a novel biochemical basis for the often found co-abuse of marijuana with such drugs. The co-abuse of marijuana with cocaine is

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Abbreviations: THC, tetrahydrocannabinol; CBD, cannabidiol; PCP, phencyclidine; MDMA, 3,4-methylenedioxyphenyl-methamphetamine HCl.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00616-5

well documented [13], and it was found to be used by 89% of patients identified as "problem" cocaine users [14], as well as in 35% of PCP abusers [15]. Furthermore, the combined use of cocaine and marijuana was identified in over 200 drug-abuse deaths [16]. More direct evidence that cannabinoids can alter cocaine effects has also been reported [17]. THC pretreatment increased cocaine plasma levels 2-fold in volunteers receiving intranasal cocaine. Furthermore, the duration and number of positive euphoric effects were found to be increased after THC pretreatment, whereas the duration of dysphoric effects was decreased. Although this study demonstrates an effect of THC on the pharmacokinetics of cocaine, very little is known of the effect of THC on other abused drugs or the mechanisms involved. Therefore, the present study was undertaken to further investigate the effects of CBD on brain levels of THC and to determine if this effect solely reflects a cannabinoid interaction or whether CBD may also affect the brain pharmacokinetics of other drugs of abuse as well.

2. Materials and methods

2.1. Materials

CBD, THC, cocaine HCl, PCP HCl, dimethyl PCP HCl, morphine sulfate, methadone HCl, and MDMA were supplied by the National Institute on Drug Abuse. All cannabinoids were prepared for intraperitoneal or intravenous injection in a Tween 80 suspension as previously described [5]; other compounds were administered in saline.

2.2. Animal treatment

Male CF-1 mice (25–35 g, Charles River Laboratories), or Sprague-Dawley rats (250–300 g) were pretreated with cannabinoids (15–120 mg/kg, i.p.) or vehicle, 1 hr (or as noted) before administration of the test compound that was to be monitored in the brain. Compounds to be monitored included THC or CBD (20 mg/kg, i.p., or 12 mg/kg, i.v., via the tail vein), cocaine (40 mg/kg, i.p.), PCP (40 mg/kg, i.p.), morphine sulfate (50 mg/kg, i.p.), methadone HCl (20 mg/kg, i.p.), and MDMA (20 mg/kg, i.p.). Doses were chosen to provide sufficient sensitivity for measurement of brain levels without undue toxicities. Animals were killed by cervical dislocation (mice) or decapitation (rats) at various time points after which brain concentrations of test compounds were determined.

2.3. Determination of cannabinoid concentrations in the brain

Brain cannabinoid concentrations were determined, as described [12,18], 45 min after administration of the test cannabinoid (THC or CBD).

2.4. Determination of cocaine and norcocaine concentrations in brain and blood

Cocaine and norcocaine concentrations in brain and blood were determined as previously described for liver [19].

2.5. Determination of PCP concentrations in brain and blood

PCP concentrations in brain and blood were determined 20–60 min after the administration of PCP (40 mg/kg, i.p.) with or without CBD pretreatment (0.1 to 120 mg/kg, i.p., for 1 hr). Brains were homogenized in 1 mL of 0.1 M potassium phosphate buffer, pH 7.4, containing 1 mM diethylenetriaminepentaacetic acid. After addition of an internal standard (20 nmol dimethyl-PCP) and 0.7 mL of 7.5% perchloric acid, the mixture was extracted with ethyl acetate, and PCP concentrations were determined after reverse-phase HPLC analysis with an Alltima C_{18} column (4.6 × 250 mm, Alltech Associates), using a water/acetonitrile gradient solvent system containing 0.1% triethylamine and 0.1% trifluoroacetic acid at a flow rate of 1.3 mL/min and UV detection at 258 nm. The gradient consisted of a linear change from 35 to 80% acetonitrile over 6 min, followed by a linear change from 80 to 100% acetonitrile over 4 min. Blood PCP concentrations were similarly determined except that 4-methyl PCP was used as the internal standard.

2.6. Determination of morphine concentrations in the brain

Morphine levels in the brain were determined 30–60 min after the administration of morphine sulfate (50 mg/kg, i.p.) with or without CBD pretreatment (120 mg/kg, i.p., for 1 hr). Brains were homogenized in 1 mL of 0.5 M sodium carbonate, pH 9.3, and, after the addition of an internal standard (5 nmol nalorphine), were extracted with ethyl acetate. Morphine brain concentrations were determined after reverse-phase HPLC analysis with an Alltima C_{18} column using a water/acetonitrile/0.1% trifluoroacetic acid gradient at a flow rate of 1 mL/min and UV detection at 220 nm.

2.7. Determination of methadone concentrations in the brain

Methadone levels in the brain were determined 30–90 min after the administration of methadone (20 mg/kg, i.p.) with or without CBD pretreatment (120 mg/kg, i.p., for 1 hr). Brains were homogenized in 1 mL of 0.1 M Tris buffer, pH 9.0, containing 3 M sodium chloride, before the addition of an internal standard (10 nmol imipramine), and were extracted with ethyl acetate. Methadone concentrations were determined after reverse-phase HPLC analysis with an Alltima C_{18} column at a flow rate of 1.2 mL/min and UV

detection at 214 nm. Elution was performed isocratically with 50% acetonitrile/0.1% trifluoroacetic acid.

2.8. Determination of MDMA concentrations in the brain

MDMA levels in the brain were determined 30–60 min after the administration of MDMA (20 mg/kg, i.p.) with or without CBD pretreatment (120 mg/kg, i.p., for 1 hr). MDMA content was determined as described for methadone content above, except for the internal standard used (100 nmol benzphetamine HCl) and elution with a linear gradient consisting of a change from 25 to 100% acetonitrile/0.1% trifluoroacetic acid over 10 min at a flow rate of 1.5 mL/min. MDMA and benzphetamine were detected at 286 and 260 nm, respectively.

2.9. Behavioral tests

2.9.1. THC

The ring test as described by Pertwee [20] was used to assay the cataleptic effect of THC. Mice were pretreated with CBD (60 mg/kg, i.p.) or vehicle for 1 hr before the administration of THC (5 mg/kg, i.p.). The time spent immobilized on a ring (5.5-cm diameter tubing clamp) after a 30-min THC treatment was determined over a 5-min time-period. Immobility was defined as the lack of locomotion (including whisker or snout movements) with the exception of respiratory movements.

2.9.2. Cocaine

Horizontal locomotor activity was determined in open field polypropylene boxes (12 × 12 × 14 inches) using a modified method of Anisman and Cygan [21]. Vehicle or CBD (30 mg/kg) was administered blindly 1 hr prior to cocaine administration (25 mg/kg). Each animal was allowed to acclimate for 20 min inside the box, which was divided into four 6 × 6 inch quadrants, before cocaine administration. Activity was determined 30 min after cocaine administration and continued for an additional 10 min. Activity was defined as the number of quadrant-crossings when at least 50% of an animal's body (excluding the tail) crossed into a new quadrant.

2.9.3. PCP

The behavioral effects of PCP were assessed by a platform-fall test as previously described [22]. Vehicle or CBD (15 mg/kg) was administered blindly 1 hr prior to PCP administration (5 mg/kg). Fifteen minutes after PCP administration, animals were placed on a 52-cm high platform (15-cm diameter with a 1.5-cm lip), and the number of animals that either fell or remained upon the platform was determined.

2.10. Statistical analysis

Statistical significance of cannabinoid-mediated changes in brain and blood concentrations as compared with vehicle-treated controls was determined by Student's *t*-test, as were the effects of CBD on THC-induced immobility in the ring test. Statistical significance of CBD-mediated effects on cocaine-induced activity as compared with vehicle-pretreated controls was determined by the Mann-Whitney U two-tailed test.

3. Results

3.1. Effect of CBD on THC brain levels

We have extended our previous studies [12] in order to characterize the dose- and time-course-effects of CBD-pretreatment on THC brain levels. We first compared the effect of the route of THC administration on CBD-mediated increases in its brain levels. Thus, although our previous study [12] employed the more technically demanding intravenous THC administration, we found in the present study that we achieved nearly identical THC brain levels when THC was administered intraperitoneally (Fig. 1), and, therefore, used this route of administration for most of the subsequent studies. Doses of CBD as low as 15 mg/kg were found to significantly ($P < 0.05$) increase brain levels of THC (Fig. 1), with a dose of 60 mg/kg increasing levels nearly 3-fold. The duration of CBD pretreatment was also found to affect the increase in brain levels of THC. The effect of CBD was time-dependent, as brain levels of THC were increased nearly 3-fold within 30–60 min of pretreatment (Fig. 2). However, when CBD was co-administered with THC (at zero time), CBD did not increase brain levels of THC significantly.

To determine whether the CBD-induced increases in THC in the brain were pharmacologically relevant, THC-induced immobility was assessed in mice by a ring test described by Pertwee [20]. Vehicle-treated control mice or mice pretreated with CBD alone were only immobile on the ring for 3–9% of the total 5-min time-period examined (Fig. 3). Mice treated with a relatively modest dose of THC (5 mg/kg) were immobilized for 35% of the time, whereas those pretreated with CBD (60 mg/kg for 60 min) before THC administration were immobilized for 74% of the time, a significant increase ($P < 0.01$).

To determine whether the effect of CBD pretreatment on brain levels of THC was species specific, Sprague-Dawley rats were similarly pretreated with various CBD doses for 60 min before THC administration. The effect of CBD pretreatment on brain levels of THC in rats was very similar to that in mice (Fig. 4). CBD pretreatment elevated THC levels in the rat brain 2- to 3-fold, with significant increases observed at a dose as low as 15 mg/kg.

3.2. Effect of CBD on brain levels of cocaine

Because marijuana is often consumed with other drugs of abuse, the influence of cannabinoids on brain levels of

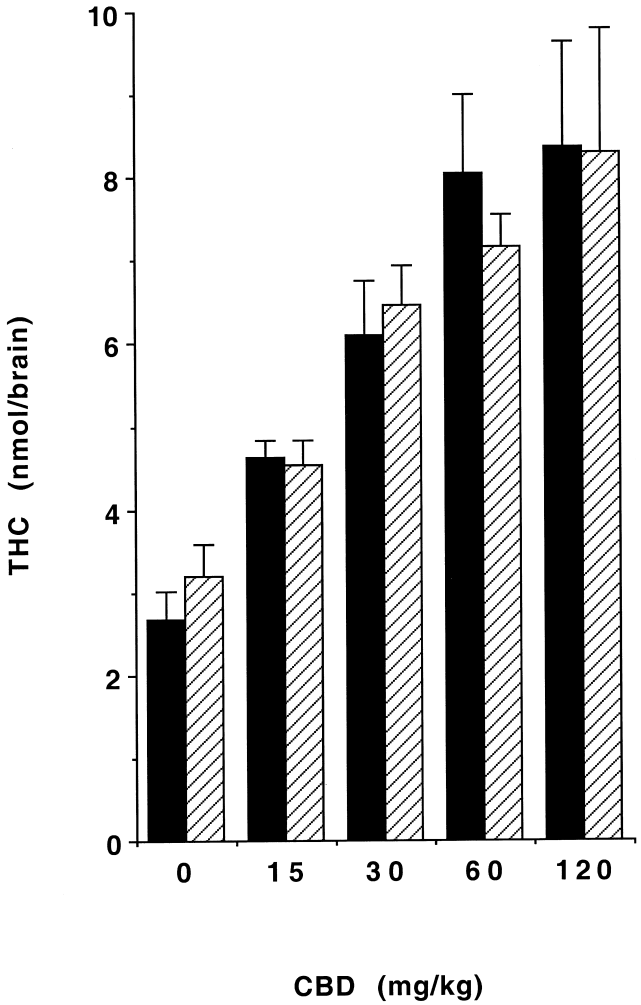

Fig. 1. Effect of CBD dose on brain levels of THC. Mice were pretreated with vehicle or CBD (15–120 mg/kg, i.p.), 1 hr before the administration of THC (20 mg/kg, i.p., solid bars; or 12 mg/kg, i.v., striped bars) for 45 min. THC content in the brain was determined by reverse-phase HPLC as described in section 2. Values represent the means ± SEM of brain levels determined in 4–6 different animals. THC levels in the brains of all CBD-pretreated animals were significantly different ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

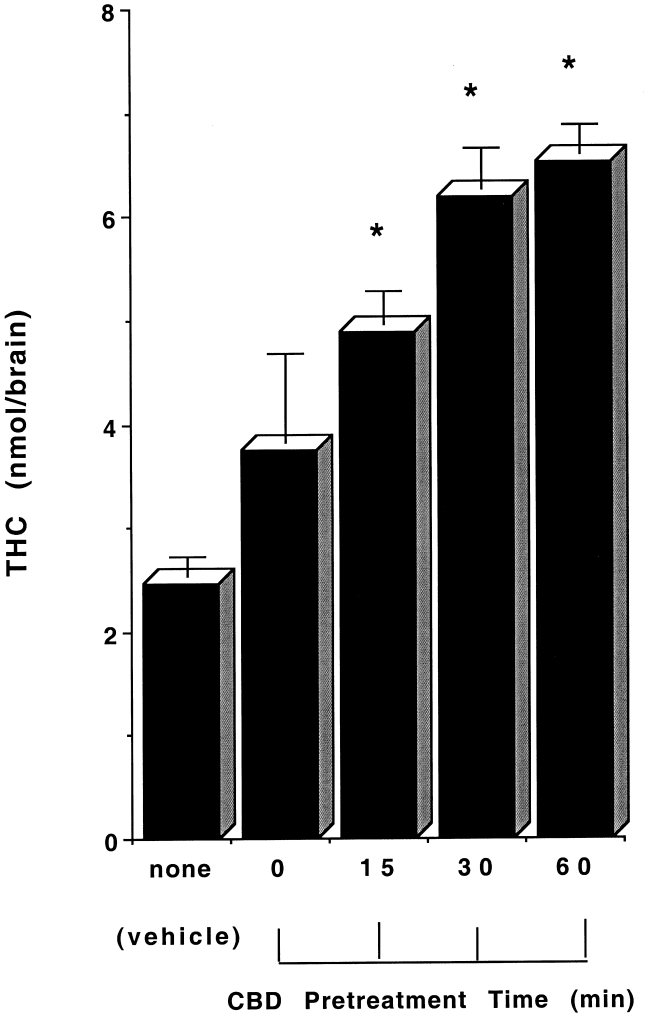

Fig. 2. Effect of CBD pretreatment time on brain levels of THC. Mice were pretreated with vehicle or CBD (120 mg/kg, i.p.), for 0 (co-injection), 15, 30, or 60 min before the administration of THC (20 mg/kg, i.p.) for 45 min. Brain levels of THC were determined by reverse-phase HPLC as described in section 2. Values represent the means ± SEM of brain levels determined in 4 different animals. An asterisk denotes a statistically significant difference ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

several other drugs of abuse was also determined. CBD doses as low as 30 mg/kg for 60 min (Fig. 5A) were able to increase ($P < 0.05$) brain levels of cocaine significantly (40 mg/kg, i.p., for 40 min). In contrast to the modest effects that CBD pretreatment had on THC blood levels [12], CBD pretreatment increased cocaine levels in the blood (Fig. 5B) to an extent (up to 3-fold) similar to those in the brain (Fig. 5A). After CBD pretreatment (120 mg/kg for 60 min), brain levels of cocaine were increased significantly (2- to 4-fold; $P < 0.05$) over those of vehicle-pretreated controls over the 20- to 60-min period examined (Fig. 5C). We also examined whether CBD pretreatment would alter brain and blood concentrations of norcocaine, since CBD is known to inactivate mouse P450 3A11, which catalyzes its formation [19,23,24]. Contrary to our expectations and as observed with cocaine (Fig. 5), CBD pretreatment actually increased the levels of norcocaine in brain (Fig. 6A) and blood (Fig. 6B) in a dose-dependent manner over the 20- to 60-min period examined (Fig. 6C). Thus, the increases in brain levels of cocaine and norcocaine could be predicted from their increased blood levels, unlike our observations with brain and blood levels of THC and its metabolites after CBD pretreatment [12].

To determine whether the increase in brain levels of cocaine elicited a corresponding pharmacological response, cocaine-induced changes in horizontal locomotor activity were assessed [21]. Mice pretreated with vehicle for 60 min before cocaine administration had an activity of 107 ± 17 (mean ± SD) quadrant-crossings, whereas mice pretreated with CBD (30 mg/kg for 60 min) had 248 ± 55 crossings, a statistically significant increase ($P < 0.01$). CBD pre-

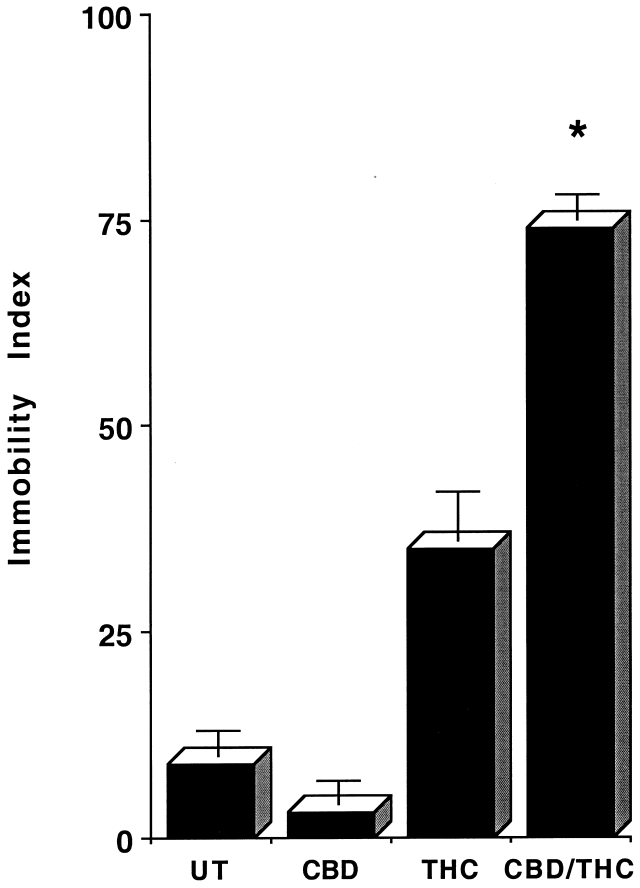

Fig. 3. Effect of CBD pretreatment on THC-induced immobility. Mice were pretreated with vehicle or CBD (60 mg/kg, i.p.) for 1 hr before the administration of vehicle or THC (5 mg/kg, i.p.) for 30 min. The immobility index is defined as the percent of time spent immobilized over a 5-min time period. Animals pretreated with vehicle before re-treatment with vehicle, pretreated with CBD before treatment with vehicle, pretreated with vehicle before treatment with THC, or pretreated with CBD before treatment with THC are identified as UT, CBD, THC, and CBD/THC, respectively. Values represent the means ± SEM determined in 6–10 different animals. An asterisk denotes a statistically significant difference ($P < 0.01$) from controls pretreated with vehicle before treatment with THC, as determined by Student's *t*-test.

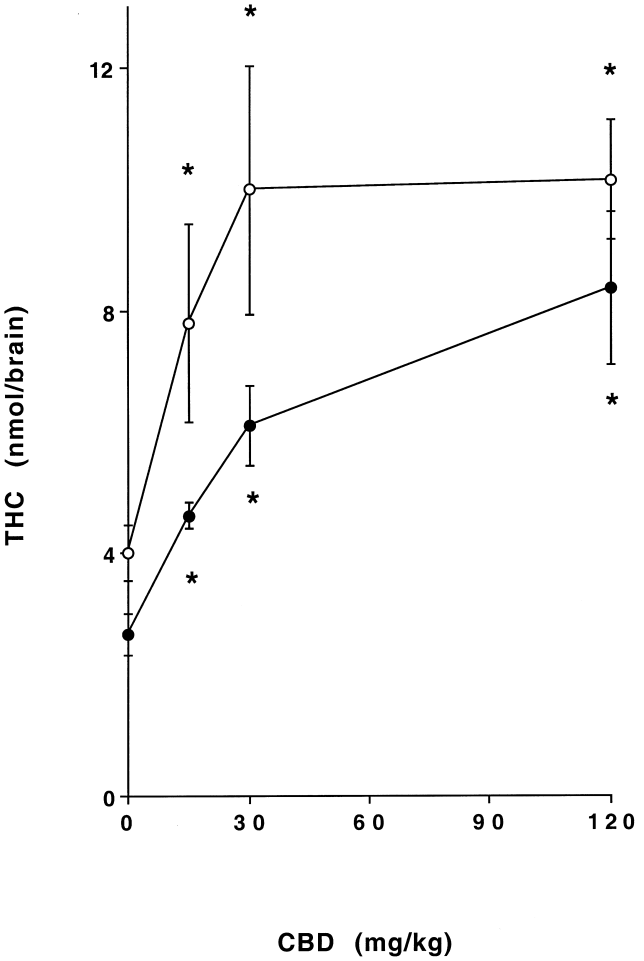

Fig. 4. Effect of CBD dose on brain levels of THC in rats and mice. Rats (○) and mice (●) were pretreated with vehicle or CBD as described in the legend of Fig. 1. THC content in the brain was determined by reverse-phase HPLC as described in section 2. Values represent the means ± SEM of brain levels determined in 4–6 different animals. An asterisk denotes a statistically significant difference ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

treatment alone had no effect on the quadrant-crossing frequency relative to vehicle-treated controls.

3.3. Effect of CBD on brain levels of PCP

To determine whether the brain levels of another drug of abuse was affected similarly to the levels of THC and cocaine, we examined the effect of CBD pretreatment on brain and blood levels of PCP (40 mg/kg for 40 min). CBD at doses as low as 1 mg/kg for 60 min was able to increase ($P < 0.05$) brain levels of PCP significantly (Fig. 7A), with PCP increases of 2- to 3-fold observed after 20–60 min of PCP administration (Fig. 7C). Blood levels of PCP were somewhat elevated after CBD pretreatment (Fig. 7B), but increases of only up to 50% were observed, similar to the magnitude determined previously with THC [12], but less than the 3-fold increase observed with cocaine (Fig. 5B).

To determine whether the increase in brain levels of PCP elicited a corresponding pharmacological response, PCP-induced platform-fall activity was assessed [22]. After a relatively modest PCP dose (5 mg/kg), only 1 of 12 mice pretreated with vehicle for 60 min before PCP administration fell from a platform. However, 9 of 12 mice pretreated with CBD (15 mg/kg for 60 min) before PCP administration fell from the platform, indicating a much higher degree of PCP intoxication. No animals pretreated with vehicle or CBD alone fell from the platform.

3.4. Effect of CBD on brain levels of opioids and MDMA

Since CBD pretreatment could increase brain levels of THC, cocaine, and PCP (Figs. 1, 5, and 7), we examined

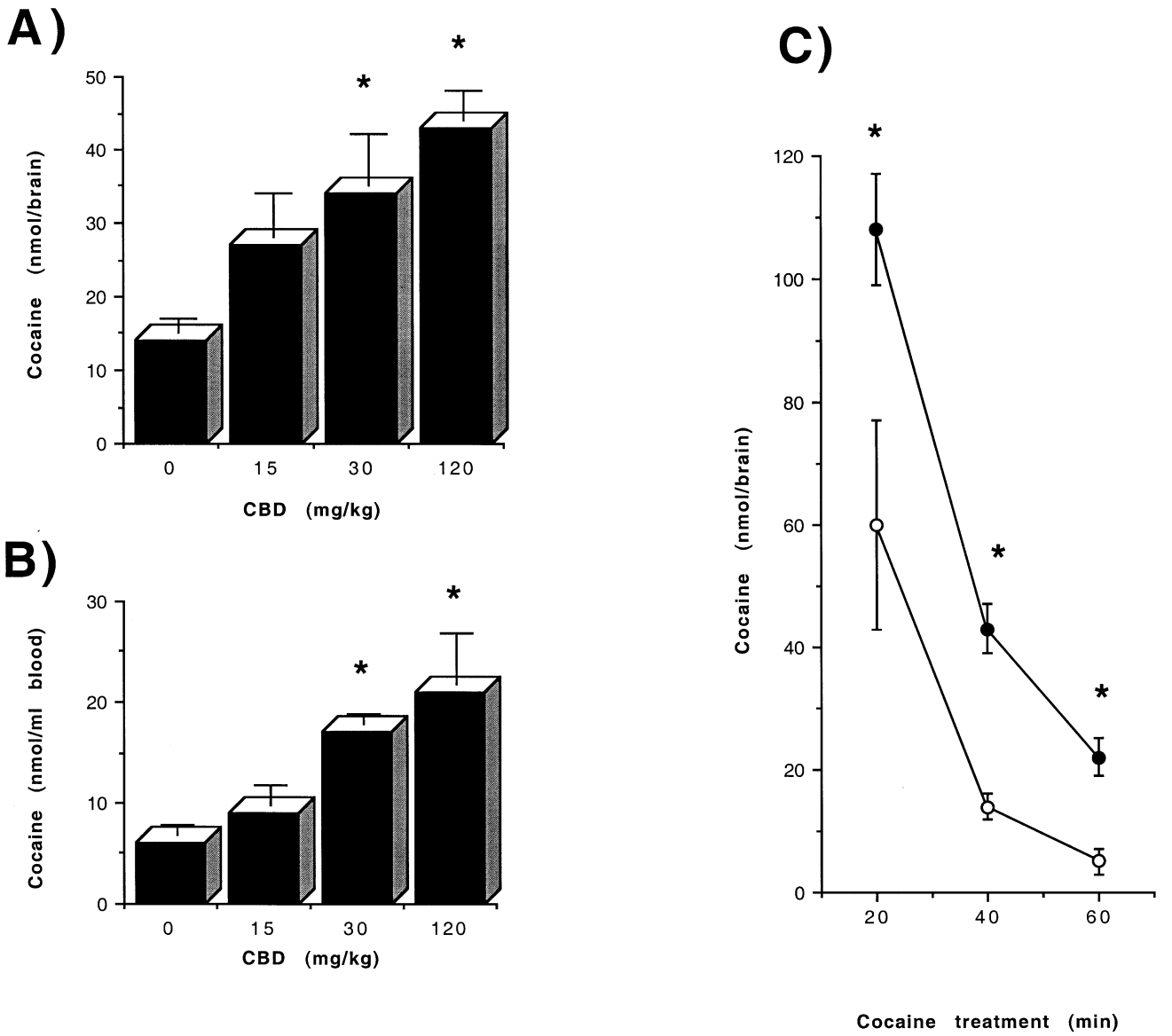

Fig. 5. Effect of CBD dose on cocaine levels in brain and blood. Mice were pretreated with vehicle or CBD (15–120 mg/kg, i.p.), 1 hr before administration of cocaine (40 mg/kg, i.p). Cocaine contents in (A) brain at 40 min and (B) blood at 20 min were determined by reverse-phase HPLC as described in section 2. (C) Mice were pretreated with vehicle (○) or CBD [(120 mg/kg, i.p., (●)], 1 hr before the administration of cocaine (40 mg/kg, i.p) for 20–60 min. Cocaine content in the brain was determined as stated above. All values represent the means ± SEM of brain or blood levels determined in 4–6 different animals. An asterisk denotes a statistically significant difference ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

whether CBD would similarly affect the brain levels in mice of other drugs of abuse such as opioids (morphine or methadone) or the hallucinogen/stimulant MDMA. CBD pretreatment (120 mg/kg for 60 min) had little or no effect on brain levels of these three drugs (Fig. 8). Preliminary experiments in which these compounds were administered intravenously yielded similar results. Thus, in contrast to the increased brain levels of THC, cocaine, and PCP observed after CBD pretreatment, brain levels of several other drugs of abuse were not similarly affected.

3.5. Effect of THC on brain levels of drugs of abuse

To determine if the influence of CBD on drug brain levels was specific for CBD itself or was a more general cannabinoid effect, mice were pretreated with THC for 60 min before CBD treatment. Under these conditions, the route of administration was critical in that after THC pretreatment, brain levels of CBD in mice administered CBD intravenously were increased, as opposed to those who received it intraperitoneally where levels did not increase (Fig. 9). THC doses as low as 15 mg/kg increased CBD

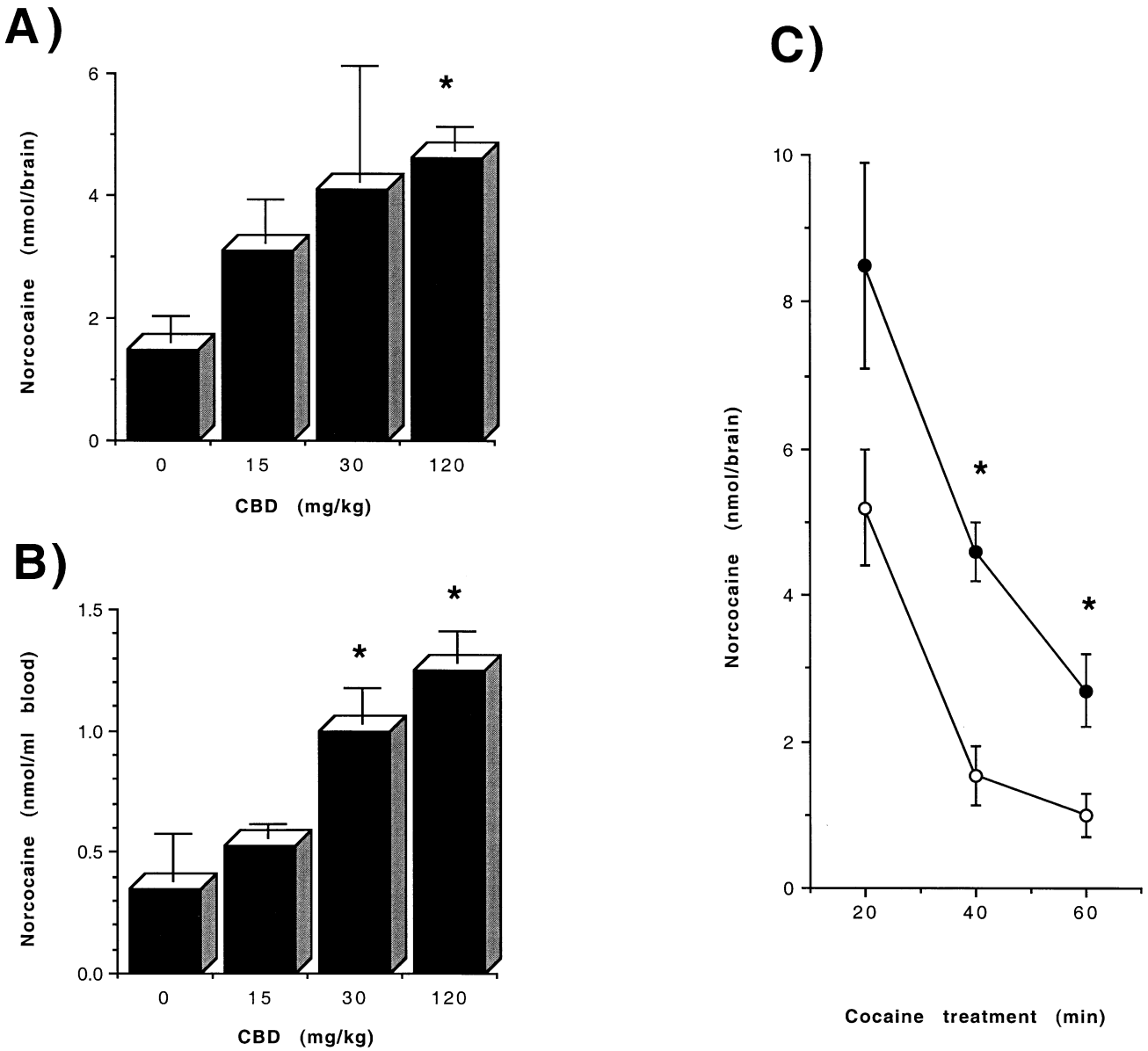

Fig. 6. Effect of CBD dose on norcocaine levels in brain and blood after cocaine administration. Mice were treated with cocaine with or without CBD pretreatment, as in Fig. 5, before the levels of norcocaine in brain (A and C) and blood (B) were determined. All values represent the means ± SEM of brain or blood levels determined in 4–6 different animals. An asterisk denotes a statistically significant difference ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

levels in the brain significantly after intravenous administration. Again, the increase in brain levels of mice that were administered CBD intravenously was nearly 3-fold after THC pretreatment, an increase comparable to that observed in brain levels of THC after CBD pretreatment (Fig. 1).

To determine if, like CBD, THC could influence brain levels of other drugs of abuse, mice were pretreated with various doses of THC for 60 min before either cocaine or PCP administration. THC pretreatment significantly increased ($P < 0.05$) brain levels of cocaine and PCP over 2-fold, but only at the highest tested dose (120 mg/kg).

4. Discussion

This extension of our previous study on the effect of CBD pretreatment on brain levels of THC [12] provides several important new findings. We have now shown that (i) CBD can exert its effect on brain levels of THC at much lower doses (Fig. 1) than the anticonvulsant dose (120 mg/kg) used previously, and (ii) manifestation of this effect requires CBD pretreatment as co-administration of the cannabinoids was not effective in altering their brain concentrations (Fig. 2). This requirement of pretreatment time

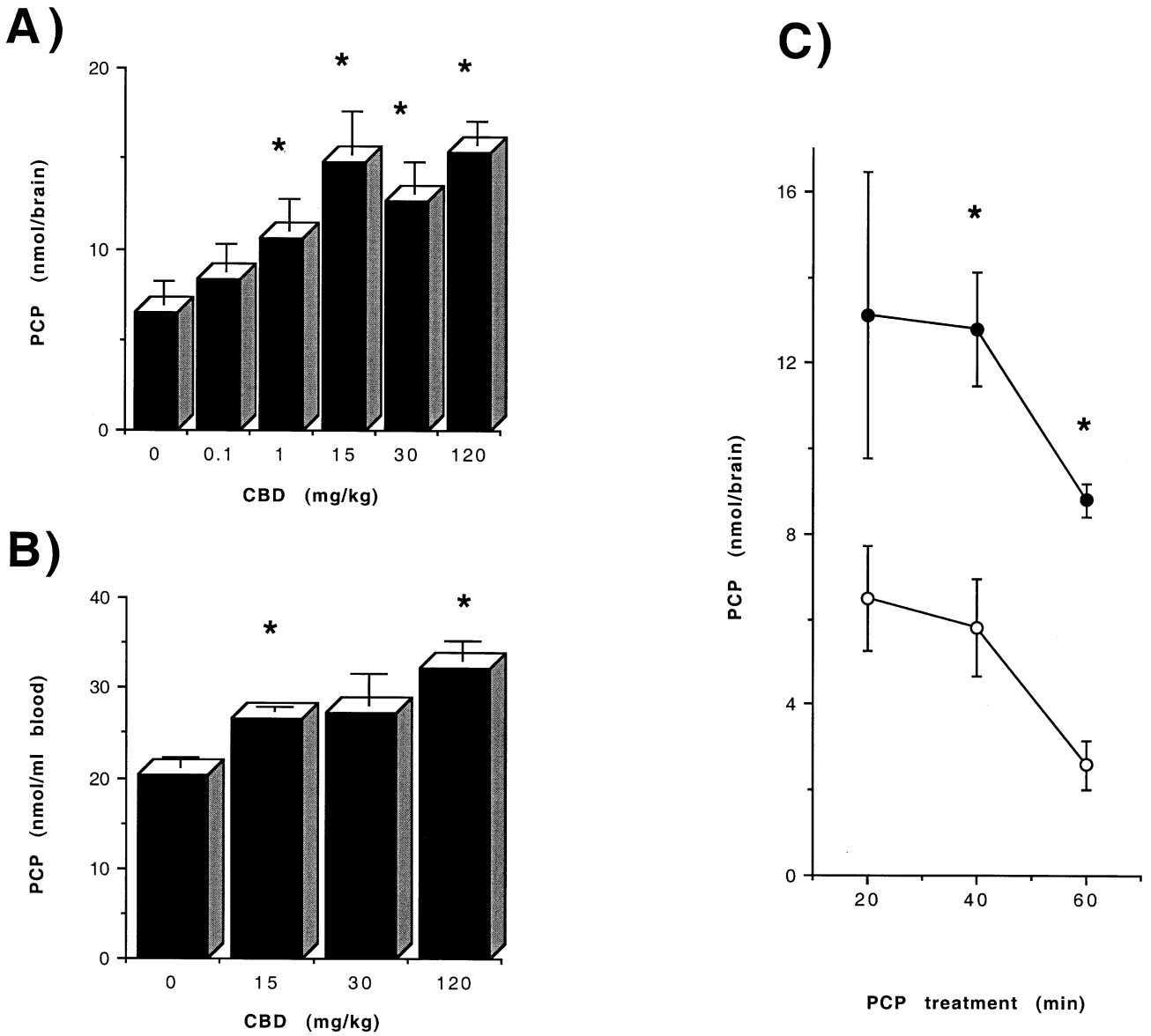

Fig. 7. Effect of CBD dose on brain and blood levels of PCP. Mice were pretreated with vehicle or CBD (0.1 to 120 mg/kg, i.p.), 1 hr before the administration of PCP (40 mg/kg, i.p). PCP contents in (A) brain at 40 min and (B) blood at 20 min were determined by reverse-phase HPLC as described in section 2. (C) Mice were pretreated with vehicle (○) or CBD [(120 mg/kg, i.p., (●)], 1 hr before the administration of PCP (40 mg/kg, i.p) for 20–60 min. PCP content in the brain was determined as above. All values represent the means ± SEM of brain or blood levels determined in 4–6 different animals. An asterisk denotes a statistically significant difference ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

suggests that either a CBD metabolite rather than CBD itself is responsible for the effect, or that CBD acts in a time-dependent process that affects brain pharmacokinetics.

CBD was used in most of these studies instead of THC for several reasons. First, since CBD is not psychoactive [3,4], it has the potential to be employed clinically to increase brain levels of therapeutic drugs whose brain distribution is limited and it does not produce the psychotoxic side-effects of THC. Second, CBD appears to be more potent in its effects on brain levels of cocaine and PCP than THC, which only increased cocaine and PCP levels in the brain at a dose of 120 mg/kg. Not only does there appear to be varying potencies of different cannabinoids, but some drugs are more sensitive to the influence of CBD than others. Thus, brain levels of PCP were exquisitely sensitive to CBD pretreatment, since a CBD dose as low as 1 mg/kg could significantly increase PCP in the brain (Fig. 7), whereas a CBD dose of at least 30 mg/kg was required to elevate brain levels of cocaine significantly (Fig. 5).

Although the pharmacological properties of THC have been studied extensively for the past 25 years [2], most studies employed THC as a single agent and not in combination with the other cannabinoids known to be present in marijuana. Since CBD and THC can mutually influence the concentration of each other in the brain (Figs. 1 and 9), it is possible that the total sum of all cannabinoids present in a

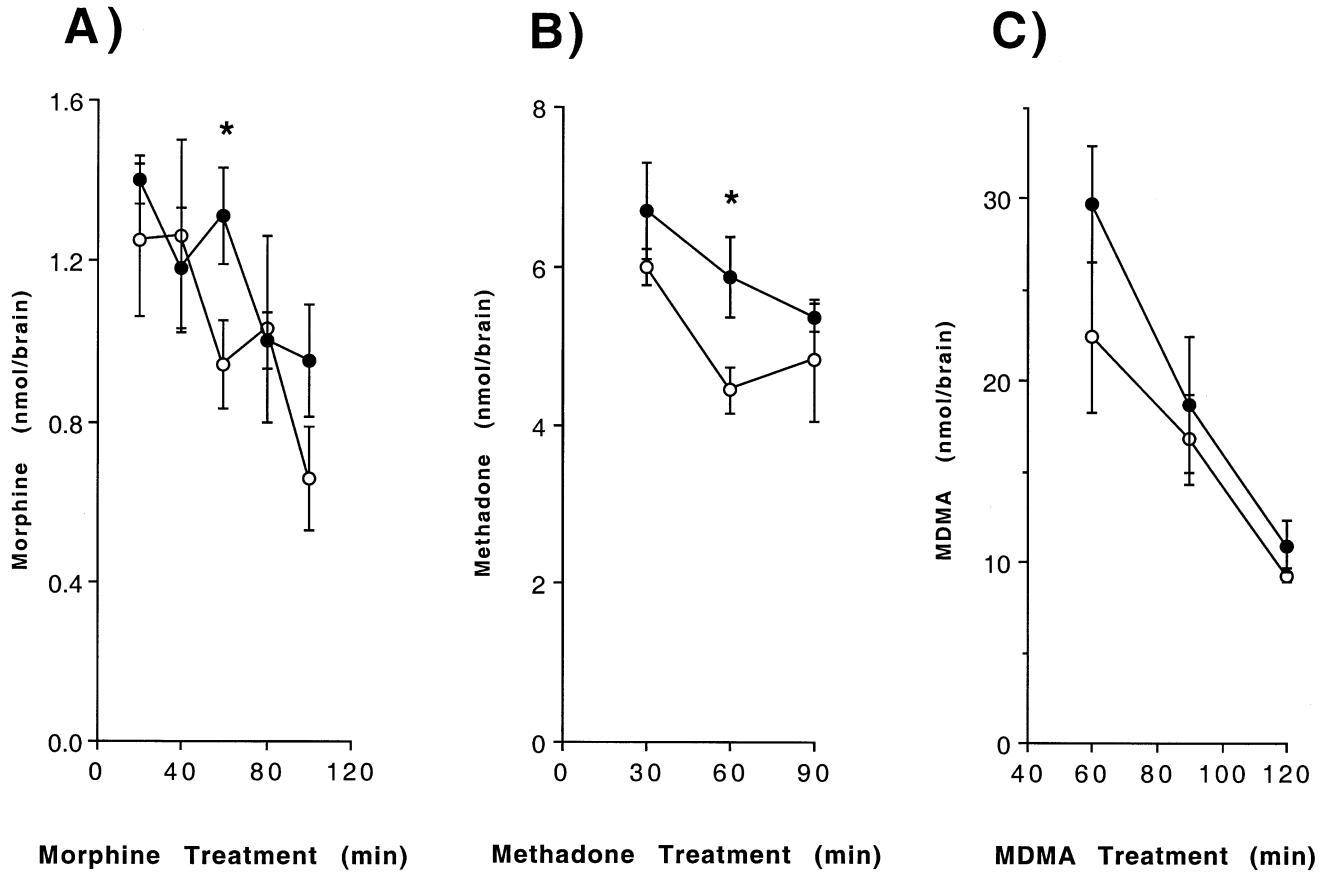

Fig. 8. Effect of CBD on brain levels of opioids and MDMA. Mice were pretreated with vehicle (○) or CBD [(120 mg/kg, i.p., (●)], 1 hr before the intraperitoneal administration of (A) morphine (50 mg/kg), (B) methadone (20 mg/kg), or (C) MDMA (20 mg/kg). Brain drug content was determined by reverse-phase HPLC as described in section 2. Values represent the means ± SEM of brain levels determined in 4 different animals. An asterisk denotes a statistically significant difference ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

marijuana sample could alter the distribution of THC in the brain and thus affect its pharmacological profile. These cannabinoid interactions may provide a biochemical basis for the greater claimed effectiveness of medically used marijuana as compared with the administration of THC alone.

It is possible and very likely that more than one mechanism is involved in the cannabinoid-mediated effects on brain levels of other drugs. Brain pharmacokinetics could be influenced by (i) effects on drug metabolism, (ii) modulation of brain transporters that transport drugs either into or out of the brain compartment, or (iii) alterations in the interaction of drugs with plasma binding proteins.

4.1. Effects on drug metabolism

Although CBD is a known P450 inactivator [5–7], we do not believe that P450 inactivation is primarily responsible for the CBD-mediated increases of brain levels of other drugs for several reasons. First, CBD pretreatment before THC administration not only failed to decrease blood levels of THC metabolites as would be expected, but, in fact, markedly increased the brain levels of such metabolites [12]. There was also no evidence for decreased cocaine metabolism after CBD pretreatment, since blood and brain levels of norcocaine were also found to be elevated rather than decreased (Fig. 6). Second, pretreatment with THC (Fig. 9), which does not inactivate P450, similarly increased not only CBD but also brain levels of cocaine and PCP, further indicating that P450 inactivation is not required for cannabinoid-mediated increases of brain levels of other drugs. Thus, although CBD can markedly inhibit P450, as determined by the *in vitro* functional assessment of several enzyme activities under conditions of saturating substrate concentrations and rate-limiting enzyme concentrations, the effects of CBD pretreatment are not consistent with the inhibition of THC or cocaine metabolism.

4.2. Modulation of brain transporters

CBD (or a metabolite) could increase active transport of other drugs into the brain or inhibit a transporter that pumps drugs out of the brain. Since CBD pretreatment failed to affect brain levels of vinblastine [25], a known substrate of the P-glycoprotein Mdr-1a brain transporter in mice [26], it is unlikely that CBD exerts its effects on brain levels

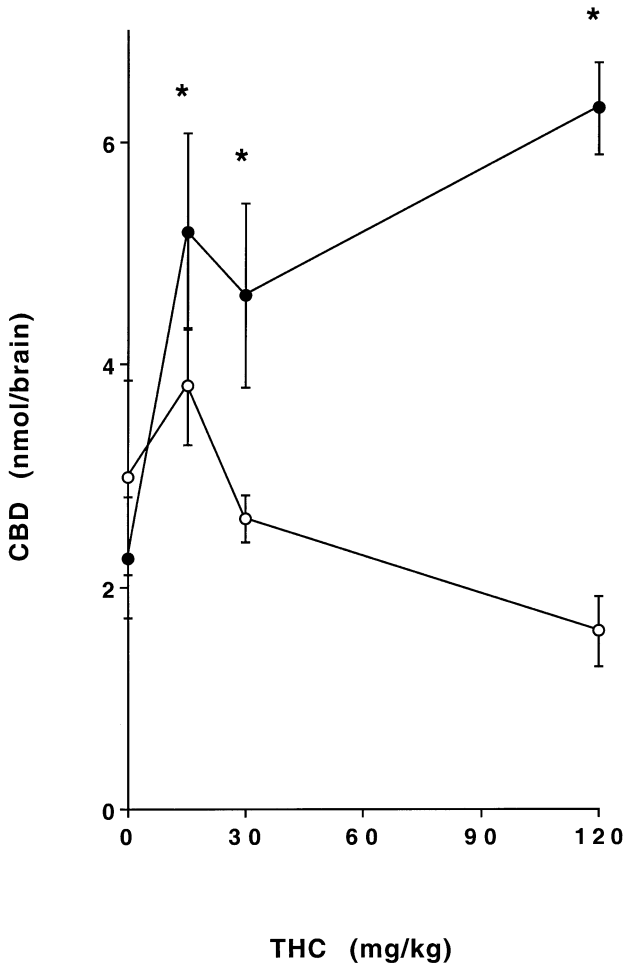

Fig. 9. Effect of THC dose on brain levels of CBD. Mice were pretreated with vehicle or THC (15–120 mg/kg, i.p.), 1 hr before the administration of CBD [(20 mg/kg, i.p., (○) or 12 mg/kg, i.v., via the tail vein, (●)] for 45 min. THC brain content was determined by reverse-phase HPLC as described in section 2. Values represent the means ± SEM of brain levels determined in 4–6 different animals. An asterisk denotes a statistically significant difference ($P < 0.05$) from the vehicle-treated controls, as determined by Student's *t*-test.

through Mdr modulation. Effects of CBD on other brain transporters are currently unknown.

4.3. Alterations in binding to plasma proteins

The brain levels of drugs are a reflection not only of their total blood concentrations, but also of their relative free or unbound fraction in blood. For drugs such as the cannabinoids that are highly bound to plasma proteins, the relative ratio of free to bound drug would be even more important. Thus, CBD (or a metabolite) could exert its influence on brain levels of other drugs by altering the fraction of unbound drug in the blood through interactions with plasma binding proteins, allowing more free drug to pass through the blood-brain barrier. Since interactions with plasma binding proteins are usually competitive in nature, a greater effect would be expected at the earliest time points of administration when CBD blood levels are the highest. Since co-administration of cannabinoids did not increase brain levels of THC, we conclude that either initial CBD metabolism is required or that the inherent time-dependent process does not involve competitive binding to plasma proteins.

The reason for the lack of a THC effect on CBD brain levels when CBD was administered intraperitoneally (Fig. 9) is presently unclear but may be due to a high first-pass hepatic clearance for CBD. Since CBD is glucuronidated to a much greater extent than THC [27,28], it is possible that only when such significant glucuronidation is delayed (as after intravenous administration, which would bypass the portal circulation) can an effect on brain levels be observed.

The effect of cannabinoids on brain levels of other drugs of abuse might have important implications for the pathogenesis of drug addiction and abuse. Since the abuse potential of a drug is greater with increasing rate of entry into the brain [29–31] and with greater maximally attained brain concentrations [29,32], it is possible that cannabinoids may play a role in increasing the abuse potential of other drugs. Thus, the cannabinoid-mediated increases in brain concentrations and pharmacological actions of several other drugs of abuse may provide a biochemical basis for the concurrent use of marijuana with other drugs of abuse. Additional clinical studies examining the effect of CBD pretreatment on THC pharmacology would be of great interest and may result in improved THC efficacy when used therapeutically. Furthermore, the consequence of marijuana use on brain levels of other therapeutically ingested drugs must be considered since not only increased effectiveness but also increased toxicities may result. It is too early to generalize about drugs whose brain levels may be affected by cannabinoids, given the limited number of drugs examined thus far. Further characterization of drugs whose brain levels may be modulated by cannabinoids will provide a more complete understanding of this phenomenon and possibly allow for a better prediction of which additional drugs may be influenced by cannabinoids.

Acknowledgments

We wish to acknowledge the use of the Liver Center Core Facility on Spectrometry (KD-26743) and to thank Drs. M. A. Correia, Department of Cellular and Molecular Pharmacology, Leslie Z. Benet, Department of Biopharmaceutical Sciences, and Peter Bacchetti, Department of Epidemiology, Division of Biostatistics, University of California, San Francisco, for invaluable discussions. This work was supported by NIH Grant DA04265 (L.M.B.).

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