ELSEVIER

Brain Research 650 (1994) 56–62

BRAIN RESEARCH

Research Report

Withdrawal from morphine or amphetamine: different effects on dopamine in the ventral-medial striatum studied with microdialysis

Donita Crippens, Terry E. Robinson *

Department of Psychology and Neuroscience Program, Neuroscience Laboratory Building, The University of Michigan, 1103 E. Huron Street, Ann Arbor, MI 48104-1687, USA

Accepted 15 March 1994

Abstract

The effect of withdrawal from chronic morphine or amphetamine treatment on dopamine (DA) neurotransmission in the ventral-medial striatum was studied by use of in vivo microdialysis. There was no effect of 24 h of amphetamine withdrawal on the basal concentration of DA in the ventral-medial striatum. Spontaneous morphine withdrawal (24 h) was associated with a significant decrease in the basal concentration of DA in dialysate, but following morphine replacement and naloxone-precipitated withdrawal variations in withdrawal symptoms were not related to variations in the concentration of DA in dialysate. It is suggested that: (1) the correlation between the extracellular concentration of DA in the ventral-medial striatum and the symptoms of morphine withdrawal may not be indicative of a necessary, causal relationship; and (2) a decrease in the extracellular concentration of DA in the ventral-medial striatum is not a common feature of drug withdrawal syndromes.

Key words: Nucleus accumbens; Striatum; Drug dependence; Drug addiction

1. Introduction

The withdrawal or abstinence syndrome associated with the discontinuation of chronic opiate drug use is quite different to that associated with the discontinuation of chronic psychomotor stimulant drug use. For example, the former includes a variety of overt neurologic and vegetative signs, as well as subjective symptoms of distress, whereas the latter is more subtle, consisting primarily of subjective symptoms [7]. However, some of the subjective symptoms associated with opiate and psychomotor stimulant withdrawal are similar, including anxiety, depression, dysphoria and drug craving. The similarity of the subjective symptoms of withdrawal across drug classes has led some investigators to suggest they may have a common neurobiological substrate [2,16,18].

Mesotelencephalic dopamine (DA) systems are known to play an important role in mediating the positive reinforcing effects of a variety of drugs of abuse [21], and it has been suggested that an increase in DA neurotransmission is responsible for the subjective pleasurable aspects of drug action (see [14] for review and an alternative hypothesis). Given this view, it is reasonable to hypothesize that a *decrease* in DA neurotransmission may be responsible for some of the unpleasant subjective symptoms associated with drug withdrawal syndromes [2,16,18]. Consistent with this idea, a decrease in the extracellular concentration of DA in the ventral striatum, as estimated by microdialysis, is reported to accompany the withdrawal syndromes associated with the abrupt discontinuation of chronic treatment with ethanol [5,16,17], morphine [1,2,13,16], amphetamine [16] or cocaine [6,10,16,20]. Some researchers, on the other hand, have reported that amphetamine withdrawal is *not* accompanied by a decrease in the extracellular concentration of DA in the ventral striatum [4,19,22]. The purpose of the two experiments reported here, therefore, was to re-evaluate the relationship between changes in extracellular DA in the ventral-medial striatum, as estimated by in vivo microdialysis sampling, and the withdrawal syndromes associated with morphine vs. amphetamine abstinence.

* Corresponding author. Fax: (1) (313) 936-2690. E-mail: GB92@um.cc.umich.edu.

SSDI 0006-8993(94)00355-G

2. Methods

2.1. Experiment 1: Withdrawal from morphine

Subjects

Thirty male Sprague-Dawley rats, initially weighing 250–300 g, were housed individually in stainless steel hanging cages. The animals had free access to food and water and were maintained on a 14:10 h light/dark cycle.

Surgery

Each rat was anesthetized with sodium pentobarbital, and standard stereotaxic techniques were used to place a 21 gauge thin-wall stainless steel guide cannula on the surface of the dura, above the ventral-medial striatum (nucleus accumbens; [12]). This was fixed in place with jeweler's screws and cranioplastic cement. The rats were allowed to recover from surgery for at least 4 days before beginning drug treatments.

Drug pretreatment and dialysis procedures

The animals were given daily injections of morphine sulfate (Mallinckrodt) or 0.9% saline for 15 days according to a regimen adapted from Acquas et al. [1]. The animals were injected subcutaneously twice per day (approximately 12 h apart) and the dose of morphine was escalated as follows: day 1, 10 mg/kg (weight of the salt); day 2, 20 mg/kg; day 3–4, 40 mg/kg; day 5–6, 60 mg/kg; day 7–8, 80 mg/kg; day 9–10, 100 mg/kg; day 11–12, 120 mg/kg; day 13–15, 140 mg/kg.

On day 15 the animals were transferred from their home cages to the dialysis test chambers. Approximately 1 h later they received one additional injection, and 2 h later each rat was briefly restrained manually while a dialysis probe (see below) was lowered into the ventral-medial striatum via the previously implanted guide cannula. The probes were perfused with a sterile salt solution containing 145 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$ and 0.2 mM ascorbic acid, pH 7.3 [8], at a flow rate of 1.5 μl/min during probe implantation. The perfusion rate was then decreased to 0.3 μl/min, and the animals were left undisturbed overnight in the test chamber.

Approximately 22 h after the last injection of morphine or saline, the flow rate through the probes was increased to 1.5 μl/min. After a minimum stabilization period of 1 h, at least three 30-min samples of dialysate were collected. After this, all of the morphine-pretreated (dependent) rats were given an injection of 60 mg/kg of morphine (s.c.), half of the saline-pretreated rats received 10 mg/kg of morphine (s.c.), and the remaining saline-pretreated rats received 0.9% saline (s.c.). A dose of 60 mg/kg of morphine was used in morphine-dependent rats because in pilot studies we determined this was sufficient to alleviate withdrawal symptoms for over 2 h. Following the injection of morphine or saline three more 30-min samples of dialysate were collected. Next, all of the saline-pretreated rats were given an injection of 1 mg/kg of naloxone (i.p.), half of the morphine-dependent rats received 1 mg/kg of naloxone (i.p.), and the remaining morphine-dependent rats received no injection. Following this, four additional 30-min samples of dialysate were collected. Thus, there were four experimental groups: (1) morphine-dependent rats given morphine only; (2) morphine-dependent rats given morphine followed by naloxone; (3) saline-pretreated rats given morphine followed by naloxone; and (4) saline-pretreated rats given saline followed by naloxone.

Dialysis probes

Concentric dialysis probes similar to those described by Robinson and Camp [15] were constructed from regenerated cellulose hollow dialysis fiber. One mm of stainless steel cannula and 7 mm of dialysis fiber extended below the skull surface. The dorsal 4 mm of fiber was coated with cyanoacrylate, leaving 3 mm of effective membrane in the ventral-medial striatum. All probes were tested for recovery of DA in vitro prior to use, and there were no group differences in recovery. The average recovery value for DA was $21.27 \pm 0.43\%$. Dopamine in dialysate was separated by HPLC and analyzed using electrochemical detection, as described previously [15].

Behavior

Behavior was monitored continuously throughout the 5-h dialysis test session, and symptoms of withdrawal were scored using scales similar to those described by Blasig et al. [3]. Each incident of the following behaviors was scored as one point: teeth chattering, wet-dog shaking, jumping, and abdominal stretching. One continuous episode of teeth-chattering was scored as one point and duration was not recorded. In addition, the presence or absence of the following symptoms was recorded every 15 min, and one point was scored if the symptom was present: diarrhea, ptosis, lacrimation and vocalization upon a light touch to the back. The total 'withdrawal score' for each animal was summed across 30-min intervals, corresponding to the 30-min dialysis sample intervals. Thus, a high score on this scale

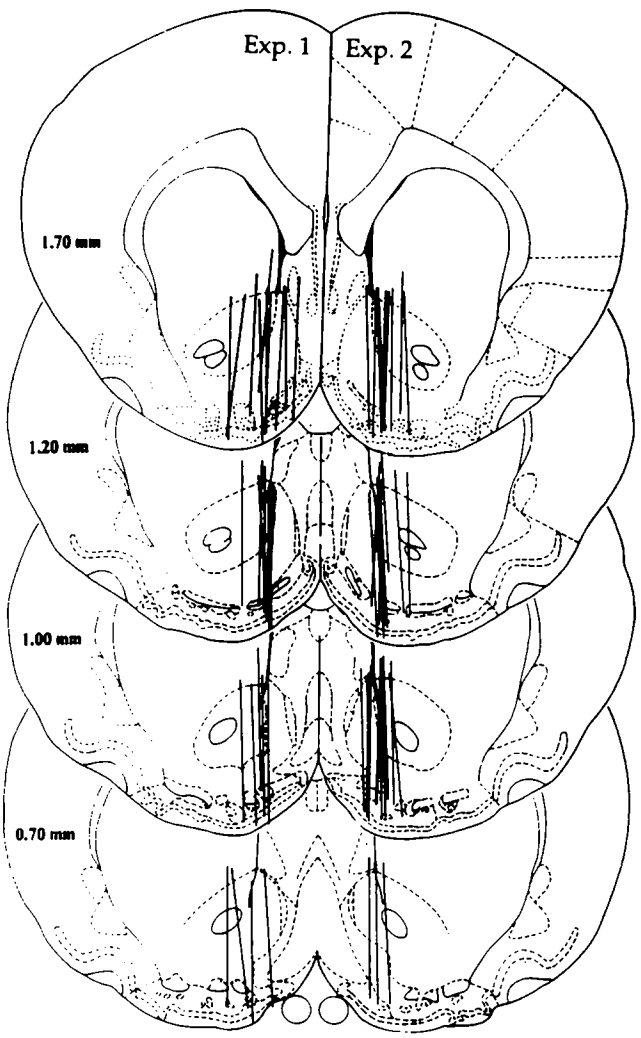

Fig. 1. Schematic drawings of coronal sections of the rat brain adapted from the atlas of Paxinos and Watson [12]. The locations of the dialysis surface of probes used in Expt. 1 (morphine withdrawal) are illustrated on the left half of the sections, and the right half of the sections shows the location of probes used in Expt. 2 (amphetamine withdrawal).

reflects the frequent occurrence of the behaviors and symptoms sampled.

Histology

Following the dialysis test session, each animal was given a lethal dose of sodium pentobarbital, and then perfused through the heart with 0.9% saline and a 10% formalin solution. The brain was removed, frozen and sectioned. The sections were stained with Cresyl violet and examined to determine the exact probe placement. Only those animals with probes that had at least 75% of the dialysis surface within the ventral-medial striatum (primarily nucleus accumbens) were used in the experiment. The neurochemical data from four animals were excluded due to incorrect probe placements or broken probes, but the behavioral data from these animals were used.

2.2. Experiment 2: Withdrawal from amphetamine

Subjects and surgery

Forty-three male Sprague-Dawley rats, initially weighing 250–300 g, were housed, and prepared with a guide cannula, as described in Expt. 1.

Drug treatment and dialysis procedures

The rats were pretreated with two injections per day, 9–12 h apart, of D-amphetamine sulfate (1.5 mg/kg, i.p., weight of the salt) or 0.9% saline for 14 consecutive days, as described by Rossetti et al. [16]. On day 15, the animals received only one injection of amphetamine or saline in the morning. Approximately 2 h later, half of the amphetamine-pretreated and half of the saline-pretreated animals were given chloral hydrate (i.p., 400 mg/kg) in three separate injections 10 min apart [16]. While these animals were anesthetized, a dialysis probe (see above) was lowered into the ventral-medial striatum via the previously implanted guide cannula, and the rats were then placed into the dialysis test chambers. The remaining rats were only briefly restrained (manually) while the probe was lowered. During the implantation procedure the probes were perfused with the same salt solution as described in Expt. 1, at a flow rate of 1.5 μl/min. Following probe implantation the pump was turned off and the animals were left undisturbed in the test chambers overnight. Approximately 23 h after the last injection of amphetamine or saline, the perfusion pump was turned on and the flow rate was gradually increased to 1.5 μl/min. After a minimum stabilization period of 1 h, at least four 30-min samples of dialysate were collected while the animals were otherwise undisturbed.

The dialysis probes were as described above, and the average in vitro recovery value for DA was 18.29 ± 0.52%. Analytical and histological procedures were as described for Expt. 1.

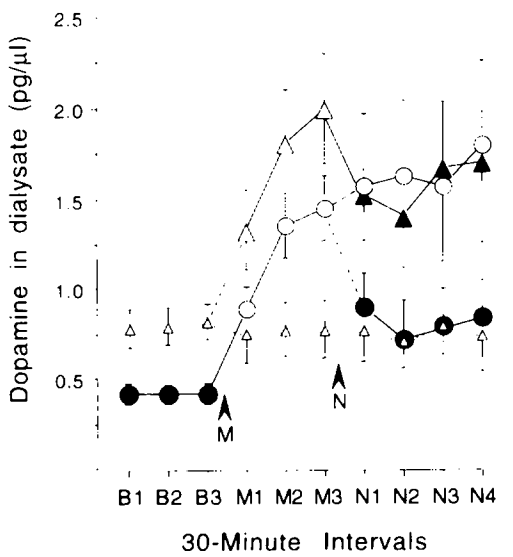

Fig. 2. The mean (+S.E.M.) concentration of dopamine (DA) in 30-min dialysate samples obtained from the ventral-medial striatum, plotted as a function of time. Values were corrected for probe recovery determined in vitro (see Section 2). Intervals B1–B3 represent basal values, intervals M1–M3 represent samples collected following morphine (or saline) administration, and intervals N1–N4 represent samples collected following naloxone (or saline) administration. Triangles represent samples obtained from saline-pretreated rats and circles represent samples obtained from morphine-pretreated (dependent) rats. All saline-pretreated rats ($n = 13$) and all morphine-dependent rats ($n = 13$) were pooled to calculate the mean basal values (intervals B1–B3). Following the collection of three basal samples, the saline-pretreated group was divided into two subgroups. The larger triangles represent those rats that received 10 mg/kg morphine ($n = 8$) and the smaller triangles represent those rats that received saline ($n = 5$). Morphine (or saline) was given at the time indicated by the arrowhead labelled 'M'. After morphine or saline administration, three additional 30-min samples of dialysate were collected (intervals M1–M3). After this, both subgroups of saline-pretreated rats received 1.0 mg/kg naloxone, at the time indicated by the arrowhead labelled 'N', and four additional samples of dialysate were collected. The black triangles represent, therefore, saline-pretreated rats that received morphine followed by naloxone. Following the collection of basal samples, all of the morphine-dependent rats received 60 mg/kg of morphine and three 30-min samples were collected (open circles, intervals M1–M3). The morphine-dependent group was then divided into two subgroups. The closed circles (intervals N1–N4) represent morphine-dependent rats that received 1.0 mg/kg naloxone ($n = 8$), and the open circles (intervals N1–N4) represent morphine-dependent rats that received no further treatments ($n = 5$). *Summary of statistical analyses.* (1) The mean basal concentration of DA was significantly lower in morphine-dependent rats than in saline-pretreated rats; comparison of the average of intervals B1–B3 for each group, $t(24) = 3.38$, $P = 0.0024$. (2) Morphine administration increased DA in both saline-pretreated ($F(3,21) = 35.1$, $P < 0.0001$) and morphine-dependent groups ($F(3,36) = 43.97$, $P < 0.0001$); one-way ANOVAs for repeated measures, intervals B3–M3). (3) Saline administration had no effect in saline-pretreated animals, and there was no change in DA concentrations throughout the test session in this group ($F(9,27) = 0.74$, $P = 0.67$). (4) In morphine-dependent animals naloxone significantly decreased DA concentrations for the remainder of the test session (one-way ANOVA with repeated measures, $F(4,28) = 8.5$, $P < 0.0001$, and follow-up Fisher's PLSD tests comparing intervals M3 vs. N1–N4). (5) In saline-pretreated animals given morphine, naloxone decreased DA, but this was not statistically significant based on a repeated measures ANOVA across intervals M3–N4 ($F(4,24) = 1.74$, $P = 0.173$); although there was a significant difference between interval M3 and the average of N1–N2 based on a paired t-test ($t(7) = 2.64$, $P = 0.033$). (6) In morphine-dependent rats given naloxone, DA concentrations did not differ from those in saline control animals (mean N1–N4 samples in morphine-pretreated rats vs. saline controls, $t(11) = 0.23$, $P = 0.82$), and were higher than during spontaneous withdrawal (average of N1–N4 samples vs. average of B1–B3 samples, paired t-test, $t(7) = 2.33$, $P = 0.053$).

3. Results

3.1. Experiment 1: Withdrawal from morphine

Histology

Fig. 1 (left) illustrates the location of the dialysis probes in the ventral-medial striatum of animals used in this experiment.

Dialysis

Fig. 2 shows the mean (±S.E.M.) concentration of DA in dialysate for each group during the entire dialysis test session. Fig. 2 shows that there was a significant decrease in the basal concentration of DA obtained from animals undergoing spontaneous withdrawal from morphine (intervals B1–B3). Indeed, the basal concentration of DA in morphine-dependent animals (0.43 ± 0.05 pg/μl) was approximately half that in saline-pretreated animals (0.81 ± 0.10 pg/μl; see the figure legends for presentation of statistical analyses).

In saline-pretreated animals, morphine administration produced a significant increase in DA concentrations (intervals M1–M3), which was reversed transiently by subsequent naloxone administration (intervals N1–N2). Naloxone alone had no effect in control animals (Fig. 2). In morphine-dependent animals the administration of morphine also produced a significant increase in DA concentrations (see Fig. 2), which was reversed by a naloxone challenge (intervals N1–N4). It is important to note, however, that following the naloxone challenge DA concentrations in morphine-dependent animals did not return to the depressed levels seen during spontaneous withdrawal, and in fact, during intervals N1–N4 DA concentrations in morphine-dependent animals did not differ significantly from those in saline control animals.

Behavior

Fig. 3 shows the average behavioral withdrawal ratings over the entire dialysis test session. Also, the average concentration of DA in dialysate is replotted from Fig. 1 for morphine-dependent animals given naloxone, and for saline control animals, to facilitate comparison of the behavioral and neurochemical data.

As expected, morphine-dependent animals exhibited spontaneous symptoms of withdrawal 1 day after their last pretreatment injection of morphine (intervals B1–B3; Fig. 3). Morphine administration eliminated the symptoms of withdrawal in morphine-dependent animals (i.e., there were no group differences in withdrawal ratings during intervals M1–M3). Naloxone administration reinstated withdrawal symptoms in morphine-dependent animals, but had no effect on saline control animals (intervals N1–N2). Naloxone-precipitated withdrawal was maximal for about 1 h after naloxone administration, but by the second hour (intervals N3–N4) withdrawal symptoms had dissipated. By the second hour after naloxone administration morphine-dependent animals did not differ from control, and also showed significantly fewer symptoms of withdrawal than at the beginning of the test session (i.e., intervals B1–B3 vs. N3–N4). Morphine-dependent animals given morphine followed by saline (instead of naloxone) did not show symptoms of withdrawal during intervals N1–N4 (data not shown).

A direct comparison of withdrawal symptoms and extracellular DA over the entire test session is especially interesting (Fig. 3). Spontaneous withdrawal was associated with a decrease in the concentration of DA in dialysate, and the alleviation of withdrawal symptoms by morphine replacement was associated with an increase in DA concentrations. Reinstatement of withdrawal symptoms by naloxone was accompanied by a decrease in DA concentrations. These results suggest a correlation between the concentration of DA in dialysate and the presence or absence of withdrawal symptoms. Fig. 3 also shows, however, clear dissocia-

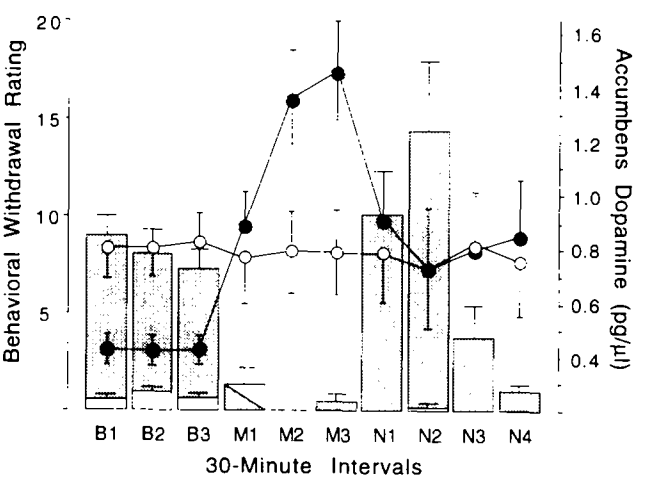

Fig. 3. Each bar represents the mean (±S.E.M.) rating of morphine withdrawal symptoms cumulated over 30-min intervals. The shaded bars represent ratings for morphine-pretreated (dependent) rats, and the open bars represent ratings for saline-pretreated rats. The closed circles represent the mean (±S.E.M.) DA concentrations in dialysate for morphine-dependent rats given morphine followed by naloxone, and the open circles DA concentrations for the saline control group. The neurochemical data are replotted from Fig. 2 to facilitate comparison of the behavioral and neurochemical data. *Summary of the statistical analyses.* (1) Morphine-dependent animals showed spontaneous symptoms of withdrawal (mean ratings over intervals B1–B3 for saline-pretreated vs. morphine-pretreated rats, Mann-Whitney U test, $U = 0.0$, $P < 0.0001$). (2) Morphine administration significantly reduced withdrawal symptoms in morphine-dependent rats (mean ratings for intervals B1–B3 vs. M1–M3, paired sign test, $P < 0.0001$), and for intervals M1–M3 morphine-dependent rats did not differ from control (Mann-Whitney U test). (3) Naloxone increased withdrawal symptoms in morphine-dependent rats for approximately 1 h (ratings for intervals M1–M3 vs. N1–N2, paired sign test, $P = 0.002$), but by the second hour after naloxone administration symptoms of withdrawal dissipated (ratings for intervals M1–M3 vs. N3–N4, paired sign test, $P = 0.34$).

Table 1
The mean ± S.E.M. concentrations of dopamine, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in dialysate (pg/μl) from the ventral-medial striatum of rats pretreated with saline or amphetamine (AMPH), and withdrawn for 24 h; half the animals were given anesthesia immediately prior to probe implantation, the day before dialysis testing

Group	Dopamine	DOPAC	HVA	5-HIAA
Saline-pretreated, no anesthesia (n = 5)	0.671 ± 0.11	573 ± 75	310 ± 42	205 ± 18
Saline-pretreated anesthesia (n = 8)	0.863 ± 0.15	621 ± 53	371 ± 38	214 ± 18
AMPH-pretreated, no anesthesia (n = 5)	0.766 ± 0.11	716 ± 55	362 ± 33	205 ± 15
AMPH-pretreated, anesthesia (n = 8)	0.637 ± 0.07	517 ± 45	285 ± 22	220 ± 17

tions between the concentration of DA in dialysate and the severity of withdrawal symptoms. First, although naloxone administration precipitated severe withdrawal symptoms (intervals N1–N2), extracellular DA only decreased to levels seen in saline-pretreated rats. That is, during the first hour after naloxone administration, when withdrawal symptoms were the most pronounced, the concentration of DA in dialysate obtained from morphine-dependent animals did not differ from control, and was significantly higher than during spontaneous withdrawal (intervals B1–B3). Second, in morphine-dependent animals the concentration of DA in dialysate was constant over the 2 h following naloxone administration, but over this same period of time the symptoms of withdrawal ranged from their maximum (intervals N1–N2) to near minimum (interval N4).

3.2. Experiment 2: Withdrawal from amphetamine

Fig. 1 (right) illustrates the location of the dialysis probes in the ventral-medial striatum of animals used in this experiment. The mean basal concentrations of DA, DOPAC, HVA and 5-HIAA in dialysate for each group are given in Table 1. There was no effect of amphetamine withdrawal, or treatment with anesthesia 24 h previously, on the concentrations of any of these compounds in dialysate, as determined by one-way analyses of variance: DA, $F(3,30) = 0.799$, $P = 0.504$; DOPAC, $F(3,30) = 2.164$, $P = 0.113$; HVA, $F(3,30) = 1.440$, $P = 0.251$; 5-HIAA, $F(3,30) = 0.199$, $P = 0.896$.

4. Discussion

Symptoms of spontaneous morphine withdrawal were clearly evident 1 day following discontinuation of chronic morphine treatment. Consistent with previous reports [1,2,16] (c.f. [13]), spontaneous morphine withdrawal was associated with a significant decrease in the extracellular concentration of DA in the ventral striatum, as estimated by in vivo microdialysis. Morphine replacement alleviated withdrawal symptoms, and increased the extracellular concentration of DA. These data are consistent with the hypothesis that a depression in extracellular DA may contribute to some of the symptoms of morphine withdrawal [2,16,18].

On the other hand, the experiment involving naloxone-precipitated withdrawal shows that the symptoms of morphine withdrawal can be dissociated from the extracellular concentration of DA, at least in the ventral-medial striatum, suggesting that the correlation described above may not be indicative of a direct causal relationship. Two examples of a dissociation between morphine withdrawal symptoms and extracellular DA were found. First, although naloxone administration precipitated severe withdrawal symptoms, the dose of naloxone used here only decreased DA concentrations to control levels; not to the depressed levels seen during spontaneous withdrawal. Second, the time course of naloxone-precipitated withdrawal symptoms was not correlated with changes in the concentration of DA in dialysate. Following naloxone administration, the withdrawal symptoms seen in morphine-dependent rats ranged from their maximum during the first hour, to near minimum during the last 30 min of the test session. Despite this marked variation in withdrawal symptoms, there was no change in extracellular DA over the same period of time. These dissociations between withdrawal symptoms and the concentration of DA in dialysate suggest that the symptoms of morphine withdrawal cannot be caused solely by variation in the extracellular concentration of DA in the ventral-medial striatum.

This conclusion requires a couple of caveats. First, although our data suggest that the symptoms of morphine withdrawal cannot be attributed *solely* to abnormally low extracellular concentrations of DA in the ventral-medial striatum, this does not mean that changes in striatal DA neurotransmission play no role in morphine withdrawal. The data only establish that variation in the symptoms associated with morphine withdrawal cannot be explained by variation in the extracellular concentration of DA *alone*. There may be a variety of complex presynaptic and postsynaptic adaptations in DA systems that occur during chronic exposure to morphine, and *interactions* between various neuroadaptations in DA systems may contribute to the morphine withdrawal syndrome. The nature of such putative interactions, and their relationship to the morphine withdrawal syndrome, remain to be determined. Second, the most obvious physical signs of withdrawal were quantified in the present study, not the aversive subjective experiences that some researchers have proposed may be mediated by changes

in DA neurotransmission [2,16,18]. It is always possible, therefore, that there was no dissociation between the *subjective* symptoms of withdrawal and extracellular DA concentrations. This would require, however, that in this experiment subjective symptoms were absent when the physical signs of withdrawal were maximal.

In contrast to the decrease in extracellular DA associated with spontaneous morphine withdrawal, there was no change in extracellular DA in the ventral-medial striatum during amphetamine withdrawal. This is consistent with a number of reports of no change in the basal extracellular concentration of DA in the nucleus accumbens over the first 5 days of amphetamine withdrawal [4,19,22]; but seems inconsistent with Rossetti et al. [16], who reported a significant decrease in extracellular DA in the ventral striatum for 5 days following the discontinuation of chronic amphetamine treatment.

It is not clear what accounts for the discrepancy between the present study (and also [4,19,22]), and that of Rossetti et al. [16]. Our previous study of amphetamine withdrawal [4], in which a quantitative ('no net flux') microdialysis method was used, differed from that of Rossetti et al. [16] in at least three potentially significant ways. First, in our previous study an escalating dose amphetamine pretreatment regimen was used, in which each of six weekly cycles of five successive drug days were followed by two drug-free days. Thus, in this experiment animals experienced withdrawal several times prior to the dialysis test session ([22] as well), whereas, in the Rossetti et al. experiment [16] animals experienced withdrawal for the first time during the dialysis test session. This raises the possibility that the response to the first experience of amphetamine withdrawal may differ from subsequent experiences. In the present experiment, therefore, we used exactly the same pretreatment regimen as Rossetti et al. [16], thus eliminating this variable. Second, Rossetti et al. [16] reported the amphetamine withdrawal-associated decrease in DA was maximal the first day following the discontinuation of amphetamine pretreatment, but in the other studies cited above animals were tested between three and five days of withdrawal. Although symptoms of amphetamine withdrawal persist for up to 7 days following the discontinuation of escalating dose amphetamine treatment [9,11], animals in the present study were examined on the first day of withdrawal to maximize the probability of detecting a withdrawal-related decrease in DA concentrations. Third, in the Rossetti et al. [16] experiment animals received anesthesia the day prior to the dialysis experiment, and in the other studies they did not. This raises the possibility that the decrease in extracellular DA reported by Rossetti et al. [16] was due to an *interaction* between residual effects of anesthesia and amphetamine withdrawal. To test this possibility in the present study, half the animals received anesthesia the day prior to the dialysis test session and half did not. Prior anesthesia had no effect on extracellular DA in the ventral-medial striatum. In conclusion, these three variables do not seem to account for the decrease in extracellular DA reported by Rossetti et al. [16].

Two other possibilities deserve mention. One, different style microdialysis probes were used in different studies. Rossetti et al. [16] used transverse probes, and concentric vertical probes were used in the present experiment, and by others [4,19,22]. The implantation of a transverse probe requires a major surgical procedure the day before the dialysis test session. It is possible, therefore, that the decrease in DA observed by Rossetti et al. [16] was not due to amphetamine withdrawal alone, but to an interaction between the discomfort associated with recent surgical trauma and that associated with amphetamine withdrawal. A second possibility is that transverse probes sample a different portion of the ventral striatum than the concentric probes used here and by others [4,19,22], and the effect of amphetamine withdrawal on extracellular DA is regionally specific. Consistent with this hypothesis, we found recently that withdrawal from escalating dose amphetamine treatment is associated with a transient decrease in extracellular DA in the dorsolateral caudate nucleus, but not the nucleus accumbens (P. Paulson and T. Robinson, unpublished observations), suggesting there are indeed regional differences in the effects of amphetamine withdrawal on DA neurotransmission.

In summary, under some experimental conditions there may be an association between the extracellular concentration of DA in the ventral striatum and amphetamine or morphine withdrawal, but the data presented here suggest this correlation probably is not due to a necessary, *causal* relationship. First, during morphine withdrawal it was possible to dissociate variation in the symptoms of withdrawal from variation in extracellular DA concentrations. Second, amphetamine withdrawal was not associated with any change in the concentration of extracellular DA in the ventral-medial striatum. Although the present study does not rule out the possibility that other, more complex, changes in DA neurotransmission play a role in drug withdrawal syndromes, it does suggest that a simple decrease in extracellular DA in the ventral-medial striatum is not a common feature of drug withdrawal syndromes, and does not play a simple causal role in producing the symptoms of opiate withdrawal.

Acknowledgements

This research was supported by a grant from the National Institute on Drug Abuse (04294). We thank

Dianne Camp and Keith Trujillo for their helpful comments and advice, and Kaitlen Browman, Hans Crombag, Steve Simmerville and Sonal Solanki for their assistance in conducting the experiments.

References

[1] Acquas, E., Carboni, E. and Di Chiara, G., Profound depression of mesolimbic dopamine release after morphine withdrawal in dependent rats, *Eur. J. Pharmacol.*, 193 (1991) 133–134.

[2] Acquas, E. and Di Chiara, G., Depression of mesolimbic dopamine transmission and sensitization to morphine during opiate abstinence, *J. Neurochem.*, 58 (1992) 1620–1625.

[3] Blasig, J., Herz, A., Reinhold, K. and Zieglgansberger, S., Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats, *Psychopharmacologia*, 33 (1973) 19–38.

[4] Crippens, D., Camp, D.M. and Robinson, T.E., Basal extracellular dopamine in the nucleus accumbens during amphetamine withdrawal: a 'no net flux' microdialysis study, *Neurosci. Lett.*, 164 (1993) 145–148.

[5] Diana, M., Pistis, M., Carboni, S., Gessa, G.L. and Rossetti, Z.L., Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence, *Proc. Natl. Acad. Sci. USA*, 90 (1993) 7966–7969.

[6] Imperato, A., Mele, A., Scrocco, M.G. and Puglisi-Allegra, S., Chronic cocaine alters limbic extracellular dopamine. Neurochemical basis for addiction, *Eur. J. Pharmacol.*, 212 (1992) 299–300.

[7] Jaffe, J.H., Drug addiction and drug abuse. In A.G. Gilman, T.W. Rall, A.S. Nies and P. Taylor (Eds.), *The Pharmacological Basis of Therapeutics*, Pegamon Press, New York, 1990, pp. 522–573.

[8] Moghaddam, B. and Bunney, B.S., Ionic composition of microdialysis perfusing solution alters the pharmacological responsiveness and basal outflow of striatal dopamine, *J. Neurochem.*, 53 (1989) 652–654.

[9] Munn, E.M. and Wise, R.A., The effects of escalating doses of d-amphetamine (AMPH) on lateral hypothalamic intracranial self-stimulation (ICSS), *Soc. Neurosci. Abstr.*, 18 (1992) 364.

[10] Parsons, L.H., Smith, A.D. and Justice, J.B., Jr., Basal extracellular dopamine is decreased in the rat nucleus accumbens during abstinence from chronic cocaine, *Synapse*, 9 (1991) 60–65.

[11] Paulson, P.E., Camp, D.M. and Robinson, T.E., The time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats, *Psychopharmacology*, 103 (1991) 480–492.

[12] Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Raven Press, New York, 1986.

[13] Pothos, E., Rada, P., Mark, G.P. and Hoebel, B.G., Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment, *Brain Res.*, 566 (1991) 348–350.

[14] Robinson, T.E. and Berridge, K.C., The neural basis of drug craving: an incentive-sensitization theory of addiction, *Brain Res. Rev.*, 18 (1993) 247–291.

[15] Robinson, T.E. and Camp, D.M., The feasibility of repeated microdialysis for within-subjects design experiments: studies on the mesostriatal dopamine system. In T.E. Robinson and J.B. Justice, Jr. (Eds.), *Microdialysis in the Neurosciences*, Elsevier, Amsterdam, 1991, pp. 189–234.

[16] Rossetti, Z.L., Hmaidan, Y. and Gessa, G.L., Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats, *Eur. J. Pharmacol.*, 221 (1992) 227–234.

[17] Rossetti, Z.L., Melis, F., Carboni, S., Diana, M. and Gessa, G.L., Alcohol withdrawal in rats is associated with a marked fall in extraneuronal dopamine, *Alcohol Clin. Exp. Res.*, 16 (1992) 529–532.

[18] Rossetti, Z.L., Melis, F., Carboni, S. and Gessa, G.L., Dramatic depletion of mesolimbic extracellular dopamine after withdrawal from morphine, alcohol or cocaine: a common neurochemical substrate for drug dependence. In P.W. Kalivas and H.H. Samson (Eds.), *The Neurobiology of Drug and Alcohol Addiction*, The New York Academy of Sciences, New York, 1992, pp. 513–516.

[19] Segal, D.S. and Kuczenski, R., In vivo microdialysis reveals a diminished amphetamine-induced DA response corresponding to behavioral sensitization produced by repeated amphetamine pretreatment, *Brain Res.*, 571 (1992) 330–337.

[20] Weiss, F., Markou, A., Lorang, M.T. and Koob, G.F., Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration, *Brain Res.*, 593 (1992) 314–318.

[21] Wise, R.A. and Bozarth, M.A., A psychomotor stimulant theory of addiction, *Psychol. Rev.*, 94 (1987) 469–492.

[22] Wolf, M.E., White, F.J., Nassar, R., Brooderson, R.J. and Khansa, M.R., Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization, *J. Pharmacol. Exp. Ther.*, 264 (1993) 249–255.