Reduction of Cocaine Place Preference in Mice Lacking the Protein Phosphatase 1 Inhibitors DARPP 32 or Inhibitor 1

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Background: *Modulation of protein phosphorylation by dopamine is thought to play an important role in drug reward. Protein phosphatase-1 (PP-1) is known to mediate some of the changes in neuronal signaling that occur following activation of the dopaminergic system.*

Methods: *Two endogenous inhibitors of PP-1 are dopamine and cyclic 3′, 5′ adenosine monophosphate-regulated phosphoprotein (DARPP-32) and Inhibitor-1 (I-1). Knockout mice lacking one or both of these PP-1 inhibitors were tested for responses to cocaine using in vivo amperometry and conditioned place preference.*

Results: *Presynaptic dopaminergic function appears to be unaffected by these mutations because stimulation-evoked changes in extracellular dopamine levels were unchanged between wild type mice and mice lacking one or both of these PP-1 inhibitors. In contrast, conditioned place preference to cocaine is reduced in mice lacking DARPP-32, I-1, or both phosphoproteins. This does not appear to be due to a learning deficit because mice lacking both DARPP-32 and I-1 show normal passive avoidance learning.*

Conclusions: *These data imply that increased PP-1 function as a result of deficits in DARPP-32 or I-1 is sufficient to decrease the rewarding properties of cocaine. Furthermore, the mechanism for this altered cocaine place preference does not involve alteration of dopamine release or reuptake.* Biol Psychiatry 2002;51:612–620

Key Words: Knockout mice, striatum, locomotor activity, dopamine, in vivo voltammetry

Introduction

Psychostimulants are thought to exert their rewarding effects through distinct cellular mechanisms that lead to increased dopaminergic transmission (Di Chiara 2000; Di Chiara and Imperato 1988; Kuhar et al 1991; Nestler 1997; Wise and Rompre 1989). In the striatum, dopamine (DA) produces its biological effects through activation of D1- and D2-like receptors and their downstream effector molecules. Protein phosphatase-1 (PP-1), a serine/threonine phosphatase distributed throughout the brain including the striatum, can modulate the phosphorylation state and activity of a variety of effector molecules that may be critical for the behavioral actions of drugs of abuse (Greengard et al 1999). Dopamine and cyclic 3′, 5′ adenosine monophosphate-regulated phosphoprotein, 32kDa (DARPP-32) is a potent inhibitor of PP-1 that is localized to striatal medium spiny neurons (Ouimet et al 1984, 1988) and regulated by phosphorylation by several protein kinases (Greengard et al 1999). Upon D1-like receptor activation, DARPP-32 is phosphorylated by PKA at threonine-34 and activated (Nishi et al 1997). In contrast, following activation of D2-like receptors, DARPP-32 is dephosphorylated through activation of calcineurin (PP2B) and inhibition of PKA (King et al 1984; Nishi et al 1999b). Hence, the activity of DARPP-32 can be regulated by dopaminergic signaling through either D1 or D2 receptors. Inhibitor-1 (I-1) is another PP-1 regulatory protein that is present in the basal ganglia, but unlike DARPP-32 it is not restricted to dopaminoceptive neurons. Inhibitor-1 is also activated by phosphorylation and is colocalized with DARPP-32 in dopaminoceptive neurons, although it is present at lower levels (Hemmings et al 1992). Inhibition of PP-1 by DARPP-32 or I-1 results in changes in the phosphorylation state and physiologic activity of ion channels, receptors, and transcription factors, resulting in altered responses to DA (Fienberg and Greengard 2000).

Given the lack of specific agonists and antagonists for DARPP-32 or I-1, mice lacking the genes encoding these

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molecules provide a model for in vivo study of the functional role of these proteins in responses to drugs of abuse. Previous studies have described the generation and characterization of mice lacking DARPP-32 (Fienberg et al 1998) or I-1 (Allen et al 2000) and have demonstrated biochemically that PP-1 regulation is altered in these mouse lines. DARPP-32 knockout mice previously have been shown to be deficient in some responses to cocaine. At the behavioral level, chronic–intermittent exposure to 10 mg/kg cocaine results in a lower rate of locomotor sensitization that is overcome at 20 mg/kg cocaine (Fienberg et al 1998; Hiroi et al 1999). Intracellular and extracellular recordings in the nucleus accumbens of DARPP-32 knockout mice have shown that medium spiny neurons from mutant animals respond less to D-1–like receptor agonists and glutamate (Fienberg et al 1998). In addition, downstream targets of PP-1 are also affected by the absence of the gene encoding DARPP-32; phosphorylation of the NR1 subunit of the NMDA-type glutamate receptor is reduced, as is the activity of the Na^+, K^+, ATPase, and the induction of chronic fos-related antigens by psychostimulants (Fienberg et al 1998; Nishi et al 1999a). The role of I-1 in dopaminergic signaling has not yet been elucidated; however, studies in brain slices indicate that mice lacking I-1 show deficient long-term potentiation (LTP) at perforant path-dentate granule cell synapses, but not at Shaffer collateral-CA1 pyramidal cell synapses. Despite this defect in perforant path LTP, I-1 knockout mice do not appear to have any spatial learning deficits (Allen et al 2000).

In our study, we used the place preference paradigm to determine whether responses to cocaine are affected by the absence of DARPP-32, I-1, or both genes. We found that mice lacking either DARPP-32 or I-1 showed attenuated place preference to cocaine and that mice lacking both genes were more profoundly impaired in their responses to cocaine. Moreover, using in vivo voltammetry, we show that this altered cocaine place preference does not involve alteration of dopamine release or reuptake.

Methods and Materials

Place Preference and Cocaine-Induced Locomotor Activity

A clear plastic cage 25.4 cm high × 50 cm long × 25.4 cm wide was used to construct the place preference apparatus, which consisted of a 25-cm square compartment with smooth white plastic flooring and white walls and a second 25-cm square compartment with black-mesh flooring and black-and-white striped (3-cm-wide) walls. A divider separated the two compartments during training sessions and a 10-cm aluminum platform was placed under the divider to discourage animals from lingering in the doorway between compartments. Cocaine place preference was performed as has been described for mice (Zachariou et al 1999, 2001). On test days, a door in the divider was raised to allow access to both compartments. To provide dim illumination and prevent interference from other environmental cues in the room, the Lucite cover of each cage was wrapped in wax paper. Movement between compartments was detected by infrared photobeam detectors and transducers set 1.5 cm above the floor of the apparatus, and activity was monitored using an IBM computer and custom software designed for measuring place preference (Cliff Moore), New Haven CT.

Tests were performed in a quiet room, and animals were moved into the room immediately before the beginning of a conditioning or testing session. On day 1, animals were weighed and placed in cages without textured floors for 5 min for habituation to handling and to the test environment. On day 2, animals were placed on the central aluminum platform and allowed to explore both compartments for 30 min to determine their initial preference between the two compartments. On days 3 through 8, animals received drug injections and were confined to the less preferred compartment (group 1: days 3, 5, 7; group 2: days 4, 6, 8) or received vehicle injections and were placed in the preferred compartment. Conditioning sessions lasted 15 min. Locomotor activity was measured during all sessions, and the three drug sessions were averaged to determine cocaine-induced locomotor activity. On day 9, the animals were placed on the central platform and allowed to explore both compartments again for 30 min. The time spent in the drug-paired compartment on the final test day was then compared with the time spent in that compartment before conditioning. Animals that spent more than 10 min on the central aluminum platform or more than 75% of the time in the preferred compartment during preference determination were excluded from the study (over all groups and doses, only 5 mice were excluded). Each animal received only one drug treatment. Cages were thoroughly washed with water and 70% ethanol between sessions.

Groups were as follows:

1. saline (IP): wild type ($n = 9$); DARPP32-/- ($n = 9$); I-1-/- ($n = 9$); DARPP32-/-/I-1-/- ($n = 8$)
2. 5 mg/kg of cocaine (IP): wild type ($n = 8$); DARPP32-/- ($n = 10$); I-1-/- ($n = 8$); DARPP32-/-/I-1-/- ($n = 9$)
3. 20 mg/kg cocaine (IP): wild type ($n = 8$); DARPP32-/- ($n = 10$); I-1-/- ($n = 9$); DARPP32-/-/I-1-/- ($n = 10$)

Passive Avoidance Learning

Ten male DARPP32-/-/I-1-/- double knockout mice and 10 wild type control mice were used for passive avoidance testing. Experiments were performed essentially as has been described (Wickman et al 2000) using a passive avoidance apparatus (Ugo Basile, Verase, Italy), which consisted of a unit with one white, lit, 10 cm × 15 cm chamber and one black, unlit, 10 cm × 15 cm chamber separated by a sliding door. The floor consisted of a metal grid wired to deliver mild foot shock of controlled intensity and duration. During shaping, each mouse was placed into the white compartment and allowed to explore both chambers of the apparatus for 5 min. During training, each mouse was put into the white chamber of the apparatus. Upon entering the dark chamber, the door closed and the mouse received a .5-mA shock of 2 sec

duration. The latency to enter the dark compartment was recorded. Following the shock, mice were removed and returned to their cage. Twenty-four hours after training, each mouse was placed into the white chamber, and the latency to enter the black chamber was measured. No shock was delivered. Mice were removed if they failed to enter the dark compartment after 5 min and were assigned a latency of 300 sec. The apparatus was wiped down with water between trials and 70% ethanol between test days. Training and testing were performed between 1 and 3 PM.

Medial Forebrain Bundle (MFB) Electrical Stimulation

Mice were anesthetized with urethane (Fluka, 1.8 g/kg, IP), fixed in a stereotaxic frame using a mouse adapter (Stoelting, USA), and body temperature was maintained at 37°C. Concentric bipolar stimulating electrodes (SNEX-200, Rhodes Medical Instruments, USA) were positioned according to a mouse atlas (Franklin and Paxinos 1997) and were implanted in the MFB 2.1 mm posterior to bregma and 1.05 mm lateral to the medial line. Their depth was adjusted for each experiment so that the DA response was maximal (ranging from 5.1–5.7 mm from the cortical surface). Stimulation pulses (.5 msec, 300 μA) were applied using an isolated stimulator (DS2, Digitimer, UK) triggered by a MacLab/2e system (ADInstrument, Castle Hill, Australia).

Electrochemical Techniques

The DA overflow evoked by MFB electrical stimulation was monitored in the striatum using carbon fiber electrodes combined with continuous amperometry as has been described (Dugast et al 1994). Measuring electrodes, the active surface of which was a carbon fiber 8 μm in diameter and 250 μm long (AGT 10 000, SOFICAR, Saint Maurice, France), were implanted in the striatum (1.6 mm lateral, 1.1 mm anterior to bregma and 2.75–3.25 mm below the cortical surface). The dura mater was punctured by a used carbon fiber electrode, and the new electrode was lowered through this hole. The reference electrode was a silver wire coated with AgCl and was maintained in contact with the skull by a sponge moistened with a .9% NaCl solution. A two electrode potentiostat (AMU 130, Radiometer Analytical, Villeurbanne, France) was used to apply +.4 V to the carbon fiber electrode versus the reference electrode and to record the current passing through them. The amplified signal was digitized by a MacLab/2e system coupled to a Macintosh computer running the Scope program (ADInstruments). Ten consecutive responses to train pulse stimulation were recorded and averaged online. Only averaged responses were stored for subsequent analysis. For each type of stimulation, the current was also recorded while the carbon fiber electrode was held at 0 V. Because at this potential DA was not oxidized, only transient electrical artifacts due to the stimulation were recorded (Dugast et al 1994). To improve the recording of oxidation currents, these artifacts were removed by subtracting data obtained at 0 V from those recorded at +0.4 V. Variations in the oxidation current were estimated in terms of changes in DA extracellular concentration on the basis of in vitro calibration of the carbon fiber electrode performed after in vivo measurement as described (Dugast et al 1994).

Data Analysis

Data for place preference, locomotor activity, and passive avoidance are presented as average ± SEM. Place preference data are presented as time in the cocaine-paired chamber posttraining minus pretraining. Locomotor activity is presented as an average of the 3 saline days and the 3 cocaine treatment days. Passive avoidance data are presented as the latency to enter the dark chamber on the training day compared with the testing day. For the place preference studies, statistical analysis was performed using repeated measures analysis of variance (ANOVA) for pre- versus posttraining with genotype as the between-group measure as well as using one-way ANOVA on the difference spent in the cocaine-paired chamber pre- versus posttraining followed by the least significant difference multiple range post hoc test, with genotype as the dependent variable. For the passive avoidance study, statistical analysis was performed using repeated measures ANOVA for pre- versus posttraining with genotype as the between-group measure. For locomotor activity studies, statistical analysis was performed using mixed-factor ANOVA with genotype as the between-groups variable and treatment as the within-group variable (saline vs. cocaine). The level of significance was set at $p < .05$.

For in vivo voltammetry experiments, two parameters were measured from evoked overflows using the Scope software. The maximal amplitude of the overflow was expressed in changes in DA concentration (nmol/L). The DA half-life corresponded to the time to 50% decay from the point where the maximal overflow was reached. The absolute values of these parameters were compared with unpaired *t* tests. The Mann–Whitney *U* score was used to compare them in drug- and vehicle-treated animals at each time after treatment.

Animals

The DARPP-32 knockout mice were selected from a colony maintained at the N_9 level of backcrossing; I-1 knockout mice were selected from a colony maintained at the N_{19} backcross level. Backcrossing was carried out onto the C57BL/6 inbred mouse strain (from The Jackson Laboratory, Bar Harbor, ME). DARPP-32/I-1 double-knockout mice: N_9 backcrossed DARPP-32 knockout mice and N_{19} backcrossed I-1 knockout mice were mated to produce individual mice heterozygous for both DARPP-32 and Inhibitor-1; these double-heterozygous mice were then mated to generate individual mice mutant for both DARPP-32 and I-1 as well as the wild type control animals. Both male and female mice were used for place preference studies (50% of each group male and 50% female). No differences were seen across gender.

Results

Place Preference

Injection of saline had no effect on place preference in mice of any of the genotypes tested (Figure 1A). No difference was seen in baseline preference in any group (not shown). The lowest dose of cocaine that could

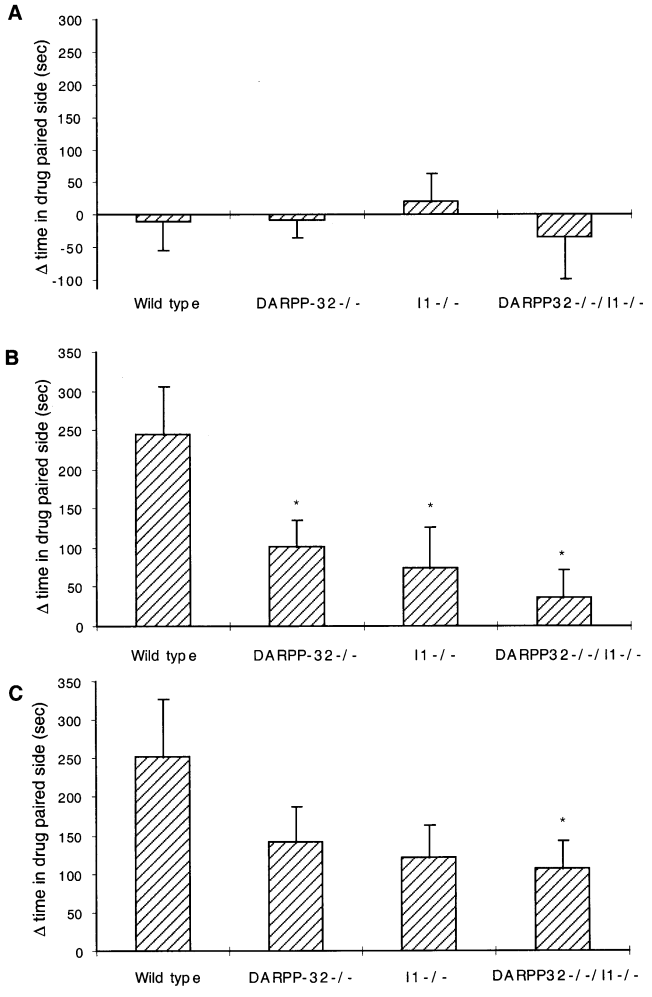

Figure 1. Cocaine place preference in mice lacking DARPP-32, I-1, or both PP1 inhibitors. Difference in time spent in the drug-paired compartment is shown following treatment with **(A)** saline, **(B)** 5 mg/kg cocaine, or **(C)** 20 mg/kg cocaine. Data are represented as difference in time (sec) spent in drug paired side post- versus preconditioning (mean ± SEM). Statistical significance was determined by one-way ANOVA on the difference scores followed by the least significant difference post hoc test ($p < .05$ for mutants vs. wild type).

condition a place preference in C57Bl/6 mice under our conditions was 5 mg/kg (Zachariou et al 1999, 2001). At that dose, wild type mice showed a 245 ± 61 sec place preference (Figure 1B). This effect of cocaine was attenuated in animals lacking DARPP-32 (101 ± 34 sec), in I-1 knockout mice (74 ± 51), and in the double mutant mice lacking both genes (36 ± 33). Cocaine at 20 mg/kg conditions a 251 ± 37 sec place preference in wild type animals, 142 ± 46 sec in DARPP-32-/- mice, 124 ± 42 sec in I-1-/- mice, and 106 ± 37 sec in mice lacking both PP1 inhibitors (Figure 1C).

Using repeated measures ANOVA for pre- versus post-training with genotype as the between-group measure,

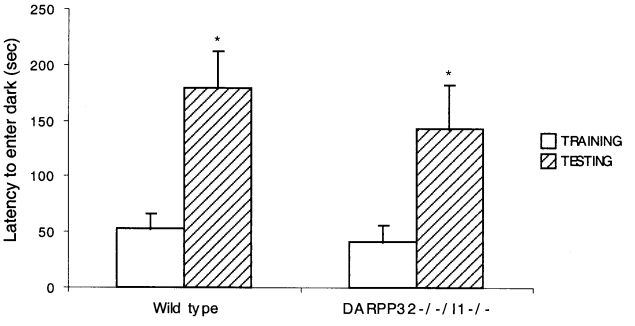

Figure 2. Passive avoidance learning in DARPP-32/I-1 double knockout mice. Latency to enter the dark chamber is shown on training day and on testing day, 24 hours after administration of foot shock (mean ± SEM). Statistical significance was determined by repeated-measures analysis of variance ($p < .01$ for training vs. testing day).

with the 5 mg/kg dose there was a main effect of training [$F(1,31) = 25.521$ and $p = .00019$]. The training × genotype interaction was also significant [$F(3,31) = 3.836$ and $p = .019$]. For the 20 mg/kg dose, there was a main effect of training [$F(1,33) = 38.148$ and $p = .0000006$], but the training × genotype interaction was not significant [$F(3,33) = 1.563$ and $p = .217$], suggesting that across genotype, there was a significant place preference to cocaine at both doses. When the difference scores are analyzed (time in cocaine-paired chamber minus baseline time in chamber) using one-way ANOVA, for the 5 mg/kg dose there was a main effect of genotype [$F(3,31) = 3.836$ and $p = .019$]. Using the least significant difference post hoc test, there was a significant difference between the wild type group and the DARPP32 -/- group ($p = .03$), the I1 -/- group ($p = .016$), and the double knockout group ($p = .003$). For the 20 mg/kg dose, there was no main effect of genotype [$F(3,33) = 1.563$ and $p = .210$] (although using the least significant difference post hoc test for wild type vs. the double knockout mice, $p = .05$).

Passive Avoidance Testing

To control for the possibility that mice lacking both DARPP-32 and I-1 were unable to associate the context with the cocaine treatment in the place preference experiment, we analyzed passive avoidance learning in these mice. No differences were seen in time to enter the dark chamber before passive avoidance training between wild type and DARPP32-/-/I-1-/- mice (Figure 2). Following training with a 2-sec, .5-mAmp foot shock, both wild type and mutant mice were able to learn the association between the shock and the dark chamber as measured by an increase in latency to enter the dark chamber post- versus pretraining for every animal in both groups [main effect of training, $F(1,18) = 26.6$, $p < .0001$]. In contrast, there was no significant difference between the behavior

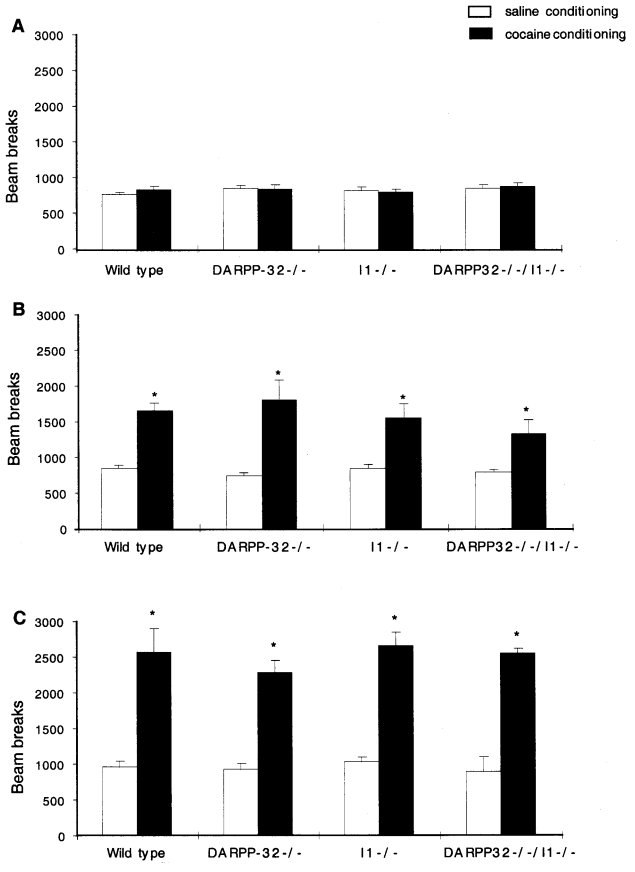

Figure 3. Cocaine-induced locomotor activity. Locomotor activity was measured during saline- and cocaine-conditioning in wild type, DARPP-32-/-, I-1-/-, and DARPP-32-/-/I-1-/- mice. **(A)** Saline treatment, **(B)** 5 mg/kg cocaine, and **(C)** 20 mg/kg cocaine are shown. Data are represented as average number of beam breaks per 15 min over 3 saline conditioning days (open bars) or 3 cocaine conditioning days (filled bars). Analysis of variance was followed by the least significant difference post hoc test ($p < .05$ cocaine vs. saline).

of the double knockout group and their wild type control animals [training × genotype interaction not significant, $F(1,18) = .307$, $p = .586$].

Cocaine-Induced Locomotor Activity

Locomotor activity was measured during conditioning sessions and averaged as a measure of saline- or cocaine-induced locomotion. No locomotor sensitization was seen over the course of the experiment. Baseline locomotor responses to saline injection were not different between the four genotypes (Figure 3A), suggesting that none of the mutations affected basal locomotor activity. In wild type mice, acute cocaine administration resulted in a twofold increase in locomotor activity. No differences were observed in locomotion induced in response to 5 mg/kg cocaine between wild type, DARPP-32-/-, I-1-/-, and DARPP32-/-/I-1-/- mice, although locomotor activity in the double knockout group tended to be lower than that of the control group (Figure 3B). Similarly, 20 mg/kg cocaine induced a 150% increase in locomotor activity that was not affected by mutation of DARPP32, I-1, or both inhibitors (Figure 3C). This suggests that actions of cocaine on locomotor activity are not regulated by DARPP-32 and I-1.

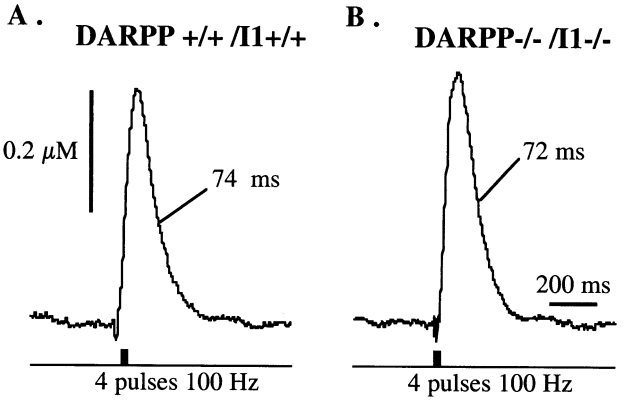

Figure 4. Dopamine (DA) overflow evoked in dorsal striatum. Typical recordings show the DA overflow evoked by medial forebrain bundle electrical stimulation (four pulses at 100 Hz) in **(A)** one wild type and **(B)** one DARPP-32-/-/I-1-/- mouse; DA half-life (time for 50% decay from the maximum) is measured in msec. The maximal amplitude of the overflow and the DA half-life are similar in wild type and all lines of mutant mice (see Table 1).

Release and Elimination of DA in Dorsal Striatum

Dopamine overflow was evoked by brief MFB electrical stimulation consisting of four pulses at 100 Hz. The DA overflow evoked by a single pulse stimulation is detectable but too small to be reliably quantified, whereas that evoked by four pulses permits quantitation (Benoit-Marand et al 2000; Garris et al 1994). Dopamine half-life did not differ between wild type, DARPP32-/-, I-1-/-, and DARPP-32-/-/I-1-/- mice (Figure 4 and Table 1) and was

Table 1. Overflow Amplitude and Dopamine (DA) Elimination in Dorsal Striatum

Mice	Overflow amplitude nmol/L	Half-life msec
Wild type ($n = 8$)	347.1 ± 54	69.6 ± 9.3
DARPP-32-/- ($n = 4$)	346 ± 73.2	65.9 ± 4.5
I1-/- ($n = 6$)	356.3 ± 73.3	64.6 ± 7.0
Wild type (double -/- controls) ($n = 5$)	350.6 ± 40.8	72.3 ± 9.3
DARPP-32-/-/ I1-/- ($n = 7$)	337.1 ± 73.2	70.9 ± 11.7

Dopamine overflow was evoked by four pulses at 100 Hz as illustrated in Figure 4. For each group, overflow amplitudes and DA half-life were measured in nmol/L and msec, respectively, and expressed as mean ± SD of *n* experiments. No statistically significant differences were observed between groups.

Table 2. Effects of Cocaine (5 mg/kg, s.c.) on Dopamine Overflow Amplitude and Half-Life

Treatment	Mice	Changes in DA half-life after injection (%)	Changes in overflow amplitude after injection (%)
Cocaine ($n = 22$)	Wild type ($n = 4$)	74.4 ± 18.4	69.3 ± 28.7
	DARPP-32-/- ($n = 4$)	87.5 ± 35.8	96.2 ± 48.1
	I-1-/- ($n = 6$)	89.1 ± 22	119.7 ± 71.4
	Wild type (double -/- controls) ($n = 4$)	70.6 ± 36.4	68.7 ± 36.6
	DARPP-32-/-/ I-1-/- ($n = 4$)	80.2 ± 24.4	95.1 ± 79.1
Saline	-/- and wild type ($n = 7$)	6.2 ± 9.4	−13.9 ± 9.0

DA overflow was evoked in striatum by medial forebrain bundle electrical stimulation consisting of four pulses at 100 Hz and four pulses at 15 Hz, applied alternately every 15 sec for 60 min. Single recordings were averaged by groups of 10 consecutive responses (every 5 min). For each group, the effects of cocaine injection on DA half-life at 100 Hz and on DA overflow amplitude at 15 Hz were measured as illustrated in Figures 4 and 5. The variations in both parameters are calculated as an average of the last three values after injection (20, 25, and 30 min) minus the average of the last three values just before injection (−10, −5, and 0 min), are expressed in percent of the last three values before injection, and are given as mean ± SD of *n* experiments.

very close to what had been previously observed under the same experimental conditions in mouse (Benoit-Marand et al 2000) or rat striatum (Suaud-Chagny et al 1995). Furthermore, no differences were found between wild type, DARPP32-/-, I-1-/-, and DARPP-32-/-/I-1-/- mice in maximal amplitude of evoked DA overflow (Figure 4 and Table 1), and these amplitudes are also very close to those previously observed in mouse striatum (Benoit-Marand et al 2000). The evoked DA overflow reflects DA release per pulse (multiplied by the number of pulses) minus DA clearance by reuptake (Benoit-Marand et al 2000; Garris et al 1994). During MFB stimulation DA clearance is minimized with the brief stimulation paradigm used. Moreover, DA clearance was identical across wild type and mutant mouse strains. Taken together, these data suggest that DA release per pulse does not differ across the mouse groups used here.

Effects of Cocaine on Evoked DA Overflow

Following cocaine injection (5 mg/kg, s.c.), DA half-life increased significantly ($p < .002$) to the same extent in wild type, DARPP32-/-, I-1-/-, and DARPP-32-/-/I-1-/- mice (Table 2). In rats and mice, dopaminergic neurons exhibit two patterns of discharge activity: single spikes and bursts of two to six action potentials (Grace and Bunney 1984; Sanghera et al 1984). Physiologic bursts of dopaminergic neurons were mimicked by MFB stimulation consisting of four pulses at 15 Hz (Figure 5A, C). Starting 10 min after injection, cocaine significantly ($p < .01$) increased DA overflow evoked by MFB stimulation (Figure 5B, C). After 20 min, DA overflow reached a plateau (Figure 5C). At this plateau, cocaine increased DA overflow with no statistically significant differences between the five groups, whereas for all groups the effect of cocaine was significantly ($p < .005$) different from saline (Table 2).

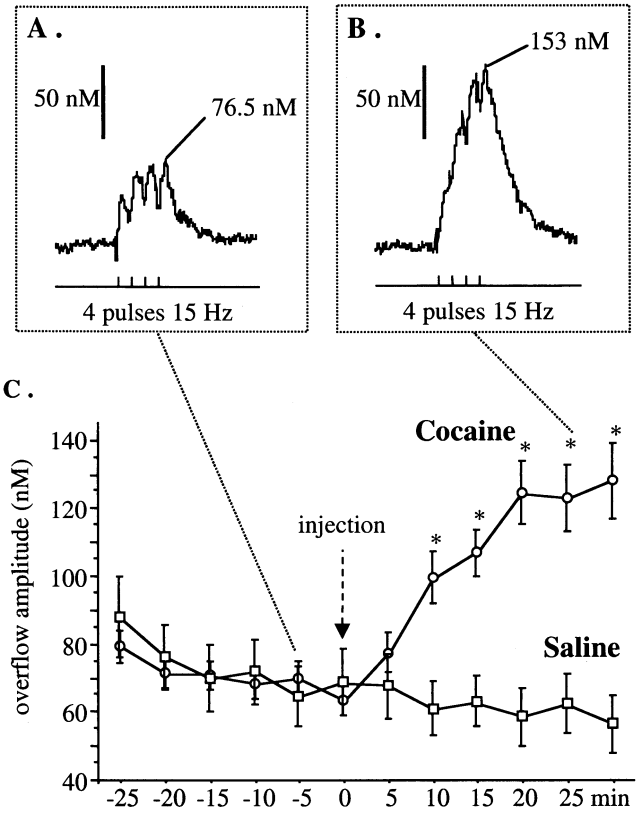

Figure 5. Effect of cocaine (5 mg/kg, s.c.) on DA overflow amplitude. We evoked DA overflow in striatum by medial forebrain bundle electrical stimulation consisting of four pulses at 15 Hz applied every 30 sec for 60 min. Recordings were averaged by groups of 10 consecutive responses (every 5 min). Typical recordings were recorded **(A)** before and **(B)** 25 min after cocaine injection from the same I-1-/- mouse. **(C)** Time course of the effect of cocaine on oveflow amplitude. Because the increase in overflow amplitude induced by cocaine is similar in all mouse groups (see Table 2), the cocaine curve shows the mean ± SEM of the 22 cocaine experiments. The saline curve was calculated (mean ± SEM) from seven control experiments performed in one mouse of each group and in two wild type mice.

Discussion

We have shown that mutation of either I-1 or DARPP-32 decreases conditioned place preference to cocaine without affecting cocaine-induced locomotion. Conditioned place preference is a behavioral paradigm that can evaluate the rewarding properties of a drug when the animal is in a drug-free state (Mucha et al 1982). We have previously shown that the threshold dose for cocaine place preference can be established using this paradigm in C57Bl/6 mice or in lines of knockout mice backcrossed onto a C57Bl/6 background (Zachariou et al 1999, 2001). Furthermore, this paradigm has been used to examine the rewarding properties of drugs of abuse in lines of knockout and transgenic mice in several laboratories (Kelz et al 1999; Martin et al 2000; Spielewoy et al 2000; Zachariou et al 2001).

Mice lacking DARPP-32 or I-1 show attenuated place preference to 5 mg/kg cocaine. When a higher cocaine dose is used (20 mg/kg), the conditioned place preference of DARPP-32-/- or I-1-/- mice, although smaller in magnitude, is not significantly different from that of their wild type siblings, suggesting that the effect of each mutation is to decrease the sensitivity to cocaine but not to abolish its rewarding properties entirely. It is unlikely that the lack of cocaine place preference to 5 mg/kg cocaine results from impairments in spatial memory because both I-1-/- mice (Allen et al 2000) and DARPP-32-/- mice (Heyser et al 2000) show normal performance in tests of spatial and associative learning and memory, although DARPP-32 knockout mice have been reported to exhibit impaired reversal learning in a discriminated operant task (Heyser et al 2000). Learning studies have not been performed previously on DARPP-32-/-/I-1-/- mice, however. Our results showing normal passive avoidance learning in the double knockout mice suggest that these animals are able to make an association between a mild foot shock and a particular context. These data, coupled with the observation that 20 mg/kg cocaine does not condition a significant place preference in the double knockout mice, suggests that normal regulation of the protein phosphatase-1 cascade is critical for cocaine reinforcement.

Behavioral responses to cocaine are not equally affected by mutation of regulators of the PP-1 cascade. Cocaine-induced locomotor activity was unaffected by targeted deletion of the genes encoding DARPP-32, I-1, or both. In a previous study, acute responses to 10 mg/kg cocaine appeared to be attenuated in mice lacking DARPP-32 (Fienberg et al 1998), but this effect was not observed in our study, perhaps because the mice we used were of a different genetic background. Other studies using knockout mice have shown a dissociation between place preference and locomotor activation by psychomotor stimulants. For example, mice lacking the dopamine D1 receptor do not show psychostimulant-induced locomotor activation (Xu et al 1994) but show normal cocaine place preference (Miner et al 1995). In contrast, D2 dopamine receptor knockout mice show impaired morphine place preference (Maldonado et al 1997) but still show locomotor activation in response to amphetamine (Chen et al 2001). These data suggest that locomotor activation and place preference may be mediated through activation of different dopamine receptor subtypes.

Cocaine is a potent inhibitor of the DA transporter, slowing DA elimination and leading to increased extracellular DA concentration (Church et al 1987; Suaud-Chagny et al 1995). This effect is thought to be important for the reinforcing and addictive properties of cocaine (Kuhar et al 1991). Although a recent study showed that mice lacking the DA transporter still self-administer cocaine (Rocha et al 1998), it is likely that, in wild type animals, the increase in extracellular DA induced by cocaine plays a major role in the rewarding effects of this drug (Caine 1998; Di Chiara 1998). The attenuation of cocaine place preference seen in DARPP-32, I-1, and double mutant mice could be due either to altered postsynaptic responses of target neurons to DA or to alterations in the presynaptic properties of DA transmission, such as decreased DA release, changes in DA reuptake, or diminished efficacy of cocaine at the DA transporter. Examination of presynaptic parameters is critical because previous studies have suggested that amphetamine-induced DA release is diminished in striatal slices of mice lacking DARPP-32 (Fienberg et al 1998). In contrast, in our study, dopamine release was monitored in vivo and evoked by more physiologic stimulation. To distinguish between these two possible mechanisms, presynaptic parameters were investigated using in vivo voltammetry. Whereas microdialysis measures the extracellular DA concentration resulting from the balance between DA release and DA elimination by reuptake, the electrochemical measurement of DA overflow evoked by MFB stimulation permits resolution of the respective contribution of release and uptake (Benoit-Marand et al 2000; Garris et al 1994; May et al 1988; Suaud-Chagny et al 1995). Dopamine half-life measured from the decay phase of the evoked DA overflow represents an accurate index of DA reuptake, whereas the maximal amplitude of the DA overflow can be used as a relative index of DA release, provided that DA reuptake is not altered (Garris et al 1994; Benoit-Marand et al 2000l Suaud-Chagny et al 1995).

The action of cocaine on addictive behaviors is thought to involve an increase in DA release, particularly in the shell of the nucleus accumbens (Di Chiara 1998), suggesting that the shell would be the ideal site for measurement of DA dynamics. Unfortunately, the small size of the shell of the nucleus accumbens in mouse results in high vari-

ability of the electrochemical measurements that is too high to permit accurate comparisons between lines of mice; however, DARPP-32 and I-1 are expressed at the same level in nucleus accumbens shell and the dorsal striatum overall (Hemmings et al 1992), making it unlikely that the lack of these proteins would differentially affect DA release.

Under basal conditions, no significant differences in DA half-life were observed in any of the lines of knockout mice examined, suggesting that DA uptake is not altered in DARPP-32-/-, I-1-/-, or DARPP-32-/-/I-1-/- mice. The amplitude of the evoked DA overflow was also unchanged in any of the lines of knockout mice. Therefore, both presynaptic aspects of DA transmission, DA release, and DA reuptake are not changed by mutation of DARPP-32 or I-1. Cocaine administration (5 mg/kg) prolonged the DA half-life and enhanced the amplitude of the evoked DA overflow to the same extent in wild type and all lines of mutant mice. Because DA overflow was evoked by stimulation mimicking physiologic bursts of action potentials, this suggests that cocaine is able to potentiate DA transmission similarly in wild type, DARPP-32-/-, I-1-/-, or DARPP-32-/-/I-1-/- mice. The effects of these mutations on cocaine place preference are therefore likely to be mediated through mechanisms postsynaptic to DA release.

Whereas mice lacking DARPP-32 or I-1 alone show similar attenuation of cocaine place preference, the double mutant mice appear to be more severely impaired. These data suggest that neither I-1 nor DARPP-32 can fully compensate for the absence of the other PP-1 inhibitor. Treatment with the D1 receptor agonist SKF 82958 increases the expression of substance P, prodynorphin, c-fos, and NGFI-A in wild type mice, an effect that is attenuated in mice lacking DARPP-32 but not in mice lacking I-1 (Svenningsson et al 2000). These data suggest that I-1 may mediate responses of drugs of abuse via mechanisms distinct from that of DARPP-32, perhaps involving different brain areas.

In conclusion, animals lacking the PP-1 inhibitors DARPP-32 and I-1 show normal DA release, normal DA reuptake, and normal cocaine-induced potentiation of evoked DA overflow but reduced sensitivity to cocaine place preference. This effect is more severe in the absence of both PP-1 inhibitors, suggesting that DARPP-32 and I-1 exert additive effects on cocaine place preference. Thus, modulation of the PP-1 cascade appears to be critical for effects of cocaine on processes downstream of DA release involved in the development of cocaine reinforcement.

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