Published in final edited form as:

Pharmacol Biochem Behav. 2015 February ; 129: 26–33. doi:10.1016/j.pbb.2014.11.021.

Quinine enhances the behavioral stimulant effect of cocaine in mice

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Abstract

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Competing interests

Authors have no competing interests

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The Na⁺-dependent dopamine transporter (DAT) is primarily responsible for regulating free dopamine (DA) concentrations in the brain by participating in the majority of DA uptake; however, other DA transporters may also participate, especially if cocaine or other drugs of abuse compromise DAT. Recently, such cocaine-insensitive low-affinity mono- and poly-amine OCT transporters were described in astrocytes which use DA as a substrate. These transporters are from a different transporter family and while insensitive to cocaine, they are specifically blocked by quinine and some steroids. Quinine is inexpensive and is often found in injected street drugs as an “adulterant”. The present study was designed to determine the participation of OCTs in cocaine dependent behavioral and physiological changes in mice. Using FVB mice we showed, that daily single injections of quinine (10 mg/kg, i.p.) co-administered with cocaine (15 mg/kg, i.p.) for 10 days significantly enhanced cocaine-induced locomotor behavioral sensitization. Quinine had no significant effect on the time course of behavioral activation. In astrocytes from the ventral tegmental area of mice, transporter currents of quinine-sensitive monoamine transporters were also augmented after two weeks of cocaine administration. The importance of low-affinity high-capacity transporters for DA clearance is discussed, explaining the known ability of systemically administered DAT inhibitors to anomalously increase DA clearance.

Introduction

Neuropharmacological studies have established an important role for the dopaminergic system in the acute reinforcing effects of drugs of abuse. Dopamine (DA) is a neurobiological substrate mediating the reinforcing effects of alcohol, nicotine, opiates and psychostimulants, such as cocaine and amphetamines (Koob and Roberts, 1998; Volkow, Li, 2005). The effect of cocaine is the most direct it has been established that the so-called “cocaine receptors” in the brain are mainly high-affinity neuronal-type dopamine transporters (DAT) (Ritz et al., 1987; Calligaro and Eldefrawi, 1988) and that cocaine acts to block the transporter, temporarily elevating extracellular DA by inhibiting its reuptake (Horn, 1990). The elevation of DA levels after cocaine administration was shown decades ago by *in vivo* microdialysis (Pettit and Justice, 1989) and cyclic voltammetry (Millar et al., 1985).

Elevation of extracellular DA is a temporary process as after some time its concentrations return to normal. The mechanism of this DA removal from extracellular space has been widely discussed in the literature, but still remains unclear. DA removal previously was mainly attributed to DAT (Ewing and Wightman, 1984; Jones et al., 1995; Wu et al., 2001). On the other hand, the same authors understand the role of extrasynaptic communication in DA transmission, in which DA is acting on spatially distinct, extracellular compartments. This implies that extrasynaptic uptake is mainly involved in rapid removal of extracellular DA (Garris et al., 1994).

Recently, low-affinity high-capacity monoamine transporters belonging to organic cation transporters family (OCT), or extracellular monoamine transporter (EMT) were characterized (Grundemann et al., 1998). Inazu et al., 2003, identified this type of transporter in astrocytes as OCT3, and others have found a splice variant for OCT1, with only partial sequence identity to OCT (Busch et al., 1998). OCTs belong to the SLC22A

subfamily and are polyspecific transporting mono- and poly-amines of wide spectrum (Sala-Rabanal et al., 2013). OCT transporters saturate at 50–100 times higher concentration of monoamines, than DAT or norepinephrine transporter (NET) (Inazu et al., 2003) and have much higher capacity at high concentrations of substrates. At low concentrations (*eg.* 100 nM) OCTs only contribute to about 20% of the DA uptake by astrocytes (Takeda et al., 2002) but their contribution increases for higher DA concentrations. Another low-affinity plasma membrane monoamine transporter (PMAT), belonging to the equilibrative nucleoside transporter family, was cloned from human brain and found in glial-like cells (Engel, et al., 2004). The multidrug and toxic compound extrusion (MATE) family of transporters can transport monoamines with low affinity and were also described in astrocyte-like cells as well (Hiasa et al., 2006). Therefore, we may conclude that low-affinity high-capacity glial transporters can play a key role in clearance of DA and other monoamines.

We previously showed (Iniouchine et al., 2008), that at high concentrations of DA, such as those usually used for *in vitro* slice electrophysiology (*eg.* 40 μ M), DA uptake depended mainly on low-affinity high-capacity transporters and was not affected by acute cocaine.

Our special interest in that study was the effect of OCT blockers on the level and the time scale of cocaine behavioral stimulant effect after acute cocaine-quinine co-administration. It is known, that quinine given at low concentrations is a blocker of OCT transporters (Bush et al., 1998; Arndt et al., 2001) and PMAT transporters (Engel et al., 2004). We, therefore, asked if quinine could interact with the extra-neuronal monoamine transporters found on astrocytes (Iniouchine et al., 2008; Inyushin et al., 2010a).

This is relevant because quinine is a known “adulterant” frequently added to heroin, cocaine and some other drugs in the street, to “cut” them. Quinine had become so regularly used for this purpose, that in some of the US states the sale of large amounts of quinine are required to be reported to the State Board of Pharmacy (Pennsylvania Pharmacy Act, 63 PS §§ 390–1 to 390–13). Quinine is believed by some drug addicts to augment the rush associated with heroin (*see.* Edwards, Augusta Chronicle, 2002). Interestingly, while quinine itself produces no addiction, the oral preference for quinine can be established in rats if for some time quinine was co-applied with cocaine (Falk et al., 1999).

Here we have shown, using cocaine/quinine co-administration in mice, that quinine facilitates the cocaine behavioral stimulant effect, while give no stimulation itself. Effects of quinine co-administration became more pronounced with prolonged used of cocaine for more than one week. Also, quinine sensitive monoamine transporter currents in astrocytes were augmented after 2 weeks of daily cocaine injections. Preliminary results were reported in abstract form (Inyushin et al., 2010a).

Materials and Methods

Animals

Age-matched FVB male mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used as the experimental subjects for behavioral studies. Adult animals were housed four per cage

in the colony room and maintained at a constant temperature and humidity with a normal-phase 12-hour light/dark cycle. They were randomly assigned to the treatment groups before the study began. Experiments were carried out with IACUC approval and according to institutional animal care and use guidelines. Adequate measures were taken to minimize pain or discomfort to experimental animals.

It is known that the acute locomotor response to cocaine in FVB mice is smaller relative to other strains, except BALB mice. FVB mice also do not show prominent sensitization to repeated doses of cocaine during the first week (Eisener-Dorman et al., 2011). In other mice strains with similar sensitivity it was shown that unlike C57BL/6J, the C57BL/6N mice has a 45% lower acute and sensitized response to cocaine and methamphetamine due to mutations in Cyfip2 protein that generally affects the amount of excitatory synapses in animals brain (Kumar et al., 2013). Others have found that low- or high-cocaine responding rat strains differ in striatal extracellular DA levels and DAT density (Nelson et al., 2009). This research shows the complexity and also the promise of studying cocaine locomotor behavioral sensitization in genetically different animals. On the other hand, the FVB strain has high reproductive success and large litters. The FVB are an inbred mouse strain, which is preferable for transgenic analyses, and is easily genetically manipulated due to prominent pronuclei in their fertilized eggs (Taketo et al., 1991). We used FVB mice in these experiments because of our future plans to use FVB/N based commercially available OCT transgenic animals (Taconic Hudson, NY, model 006622), and because FVB mice can be rapidly bred in our animal facility.

Drug concentrations

We used a 10 mg/kg concentration of quinine because we were aiming to mimic the ratio of cocaine-quinine found in “street drugs”. The mean concentration of cocaine in street drug samples varies with years (Cunningham et al, 1984; Schneider, Meys, 2011) but stays between 43% to 64%. In our work we used 60% cocaine, and 40% quinine mix in our experiments. Also, we did additional experiments with intraperitoneal injections of 10 mg/kg quinine and found it has no clear behavioral effect (there was some limited inhibition of ambulatory behavior but without statistical significance). Also, from our previous experience we know that this concentration of quinine (10 mg/kg $\approx$ 30 μ M) significantly reduces dopamine uptake by low affinity transporters in the brain, but only has limited or negligible effects on potassium currents (Iniouchine et al, 2008).

Peakman et al, 2003, and Zombeck et al, 2010 proved that 15 mg/kg i.p cocaine is sufficient to produce a behavioral stimulation effect and sensitization in the FBV mouse strain used in our study. This lead as to use 15 mg/kg cocaine and 10 mg/kg quinine in our experiments

Behavioral testing

Behavioral experiments were conducted at the Behavioral Testing Facility of the Universidad Central del Caribe (<http://www.uccaribe.edu/btf/>). Measurements were made at room temperature (22–24°C) in an appropriately lighted room during daylight hours. Test animals were placed inside a plastic test enclosure (each animal tested in a separate enclosure) within an acoustic chamber to mask external noises and other perturbations. Mice

movements in the test enclosure were measured and evaluated using a video camera connected via a digital video board to the computer using Ethovision® software (version 3.0; Noldus Information Technology, Leesburg, VA, USA).

Mice were randomly assigned to two experimental groups (6 animals per group) that were treated daily for 10 days with either 15 mg/kg of cocaine, or 15-mg/kg cocaine plus 10-mg/kg quinine, as a single daily i.p. injection, to establish the development of cocaine behavioral sensitization. The third group were administered isovolumetric quinine injections over 10 days and served as a control. A fourth group, which served as a vehicle (saline) control group, was also tested. Each group was habituated to the behavioral testing procedures by administering a saline injection prior to being placed into the test enclosure and monitored by the video camera to measure locomotion for 5 days prior to beginning of the experiment. In addition, before each injection the animals were habituated to the test enclosure for 1 hour. Mice were then injected with their respective drugs and returned to the testing chamber for 2 more hours during which their locomotor activity was analyzed. Locomotor activity was recorded as distance moved in cm during 1-min intervals following the daily injection. Cocaine hydrochloride and quinine hydrochloride dihydride were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA) and were diluted in saline. Doses are expressed as the weight of the salts.

Preparation of brain slices

Adult mice were decapitated and the brains were removed from the skull in ice-cold (2°C–4°C) dissecting solution composed of (in mM): 126 NaCl; 2.5 KCl; 1.2 NaH₂PO₄; 7.0 MgCl₂; 0.5 CaCl₂; 25 Glucose; 25 NaHCO₃; saturated with 95% O₂ and 5% CO₂. Brain tissues were sectioned and 250 µm slices were cut using a vibroslicer (Leica VT 1000S, Leica Microsystems, Wetzlar, Germany). The slices were placed in an incubation chamber containing oxygenated artificial cerebrospinal fluid (ACSF) (in mM: NaCl 126; KCl 2.5; 1.2 NaH₂PO₄; 1.0 MgCl₂; 2.0 CaCl₂; 25 Glucose; 25 NaHCO₃; saturated with 95% O₂ and 5 % CO₂) at 35°C and incubated at least 60 min before recording. Then, slices were placed in the recording chamber (500 µL) and superfused with ACSF (35°C) at a rate of 2 mL/min. During transporter-associated current measurements Ba²⁺ (100 µM) was added to the ACSF to reduce potassium currents and to avoid the influence of K⁺-channels (Brew and Attwell, 1987)

Whole-cell recordings

As described earlier (Inyushin et al., 2010) the recording area of a brain slice in midbrain ventral tegmental area (VTA) was identified visually with an Olympus BX51WI microscope equipped with an infrared light differential interference contrast (DIC) system and a 40×-water immersion objective (Olympus Corporation, Center Valley, PA, USA). Whole cell recordings were made with borosilicate glass pipettes with tip resistance of 5–6 MΩ when filled with the internal solution of (in mM) 115 K-gluconate; 20 KCl; 1 MgCl₂; 10 HEPES; 0.1 EGTA; 2 ATP; 0.3 GTP; 10 creatine phosphate. Additionally, 10 µM of cocaine was added to the solution to block cocaine-sensitive transporters. After gigaseal formation, the holding potential was set to 85 mV and the fast capacitance compensated. Whole cell recordings were obtained using the MultiClamp-700A amplifier (Axon Instruments, Union

City, CA, USA) and membrane currents were filtered at 1 kHz and digitized at 5 kHz (Digidata 1322A, Axon Instruments). Electrode potentials were compensated automatically, using MultiClamp-700 software features. Astrocytes were first recognized under DIC visually by shape and size, and then identified electrophysiologically using a standardized puff-application of 4-(4-(dimethylamino)-styryl)-N-methylpyridinium (ASP⁺) as described in (Inyushin et al., 2012).

To distinguish between neuronal and non-neuronal type transporters, we applied elevated concentrations of ASP⁺ substrate in order to take advantage of the kinetics of the high capacity of non-neuronal monoamine transporters and differentiate them from the high affinity low capacity transporters such as NET.

Statistical Methodology

For co-administration of cocaine and quinine, we used a ten days (D1-D10) statistical model with three comparison groups as follow: *control*, *cocaine only* and *cocaine + quinine*. First, comparisons were made between each day independently with the comparison groups in order to find statistical significance at a bivariate level. To account for the time horizon as a whole statistical unit, a multivariate analysis was performed to calculate estimated marginal means. To evaluate the nature of all the variables in our dataset, normality diagnostic was done using the Shapiro-Wilk estimator. A One way ANOVA with Bonferroni post hoc test was performed in order to establish comparisons between study groups and days as independent units.

To study a general trend of behavioral activation changes in experimental groups a General Linear Model Repeated Measures ANOVA approach was used controlling the inherent variations in the model in terms of the fluctuations over time to calculate estimated marginal means taking into account all ten days as a unit. A Mauchly's test of sphericity was performed to assess if our models has or not the assumption of compound symmetry. If non-significant, we report the univariate results with an Epsilon correction; if significant, we report the multivariate results using the Pillai's Trace estimator. Either of the last explained results was used to evaluate the time effect in our model.

A test of Between-Subjects Effect with a pairwise comparison (Sidak post-hoc adjustment) was applied to perceive statistical differences between and within the groups. The estimated marginal means will be reported. The significant level (α) was set to 0.05. IBM Statistical Package for Social Sciences (IBM-SPSS, Chicago, IL) v.22.0 for Windows was used.

An unpaired t-test was used to find statistical difference between two groups in additional experiments. Calculations were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA).

Results

Quinine enhanced cocaine-induced behavioral activation in mice

Behavioral experiments demonstrate that daily single injections of quinine co-administered with cocaine for ten consecutive days enhanced cocaine-induced behavioral activation in

mice. Both experimental groups (cocaine-only and cocaine-quinine) demonstrated sensitization of locomotor activity, but in the “cocaine-quinine” group the sensitization of locomotor activity was dramatically increased and achieved its maximum. In total, 28 animals were used in the main and additional experiments.

Figure 1 shows the group average distance moved per min (cm) during the first 30 min after injection (mean $\pm$ SEM). Daily i.p. injections of cocaine alone (15 mg/kg; cocaine group, closed squares), or cocaine (15 mg/kg) co-administered with quinine (10 mg/kg; cocaine + quinine group, closed circles) for two consecutive weeks produced increasing amounts of locomotor activity over the 10 days of injections. Locomotor activity in both the cocaine and the cocaine + quinine groups was significantly greater than seen after administration of quinine alone (15 mg/kg, control group, open circles) on all days. Quinine, co-administered with cocaine, significantly enhanced cocaine-induced behavioral sensitization in mice during the last 5 injection days (asterisks). Significant effects were determined by one-way ANOVA, and Table 1 shows the statistical results for separate days, ($p < 0.05$).

Repeated administration of cocaine to rodents has previously been shown to produce a progressive augmentation in motor activity known as behavioral sensitization (Downs and Eddy, 1932; Stripling and Ellinwood Jr., 1977; Kalivas et al., 1992). Over the first 30 minutes, there was a general trend for increasing locomotor activity in the two groups that received cocaine repeatedly across the 10 days of injections. In contrast, mice that received repeated injections of only quinine did not show progressive changes in locomotor activity.

Differences between the “Cocaine + Quinine” and the “Cocaine” group emerged during the second week (Fig. 1, asterisks last five days). In order to show statistically the general trend of changes in behavioral activation, we used a Repeated Measures ANOVA that had shown a large statistical difference between behavioral activation of experimental and control groups. Sphericity assumption was met (Mauchly's $W = 0.102$, $p < 0.05$). There was a significant effect in terms of mean change throughout time (Pillai's Trace $F = 69.52$, $p < 0.001$), the test of between-subjects effect with a pairwise comparison proved significant ($p < 0.001$). Statistically differences were noticed between treatments ($F = 11.81$, $p < 0.001$) (Control $EMx = 83.60$; cocaine only $EMx = 412.07$, cocaine and quinine $EMx = 393.43$). Pairwise comparisons with a Sidak correction (Sidak post hoc) shows statistical differences in control vs. cocaine alone and control vs. cocaine and quinine, and most importantly between the cocaine only and the cocaine and quinine group ($p < 0.05$). The average of **distance moved** of the mice exposed to *cocaine + quinine* tended to be greater over time than the mice exposed to the other two treatments, the average level of **distance moved** in the mice exposed to *cocaine + quinine* tended to stabilize after D6.

Additional control: to find out if quinine alone produced any increased locomotor activity, we compared the group average distance moved during the first 30 min for animals that received repeated injections of quinine (10 mg/kg; control group) with another group that received repeated saline injections (4 animals per group, total 8 animals) under the same conditions previously described. While there was some inhibition of mean activity in quinine treated animals (159.3 ± 11.87 versus 184.3 ± 11.67 in saline control, $t = 1.588$ $df = 18$, $p = 0.13$), there was no significant difference.

Quinine only affected the amplitude of cocaine's locomotor stimulating effects, but did not significantly change the temporal course of the cocaine-induced behavioral activation in mice

We compared the time-course of the development of behavioral sensitization by examining graphs showing the average distance traveled (cm) during each of the 120 min of the behavioral observation session (Fig. 2). In the upper left-panel, day 1, it is evident that the groups receiving cocaine, or cocaine + quinine, exhibited elevated behavioral locomotor activity compared to the control (quinine only) group. The distance traveled for most minutes was doubled or more of the control group and the elevated locomotor activity was sustained for the duration of the 2-hr session. By day 2, the development of sensitization (during the early parts of the session—distance traveled during the first 30 min) increased from approximately 300 to 400 cm/min between day 1 and 2. By the day 3, this initial burst of increased activity during the first 30 min increased to about 430–530 cm/min in the cocaine treated groups. Across the first three days, the cocaine effects are larger and sustained across the 2 hr session.

This pattern of changes in the behavioral response to cocaine and cocaine + quinine continues through the 10 day course of the experiment. The initial burst of activity during the first 30 min following cocaine and cocaine + quinine continues to get progressively larger, but the effects of cocaine + quinine separate out and become larger than the effects of cocaine alone. The duration of the increased locomotor activity, compared to the control group, remains the same, at least as can be observed during the 2 hr session length.

Monoamine transporter currents in astrocytes are increased by chronic administration of cocaine

Behavioral data (Fig. 2) suggest that cocaine and quinine produced long lasting effect on locomotor activity probably via increasing DA transporter efficiency in glial cells. Therefore, we tested electrogenic OCT transporter currents in these animals. Using whole-cell patch-clamp recording from astrocytes in VTA brain slices, we measured inward currents generated by ASP⁺ (Fig. 3). ASP⁺ is a substrate for both (i) cocaine-sensitive high-affinity neuronal monoamine transporters such as NET, DAT and SERT, and also for (ii) the OCT-type quinine-sensitive low-affinity monoamine transporters (Schwartz, 2003; Çetinkaya et al., 2003). Here we used a high concentration of ASP⁺ (10 μ M), established as the substrate for OCTs in astrocytes and to ensure that ASP⁺ will be transported primarily via non-neuronal type transporters in glia (Iniouchine et al., 2008; Inyushin et al., 2010b). This is because at high concentrations of substrates, the low-affinity and high-capacity transporters such as OCTs, have a kinetic advantage. We also used ASP⁺ with quinine as a mixture to see if transporter-associated currents were sensitive to quinine. These transporter currents were quinine-sensitive (Fig. 3) in both control (saline-treated, panel A) and cocaine-treated mice (daily injections of 15 mg/kg for 10 days, panel B). Furthermore, the magnitude of ASP⁺-induced current was much greater in astrocytes of cocaine-treated animals (122 ± 16 pA ($n=6$)) as compared to control (53 ± 12 pA ($n=6$)). This significant ($t=6.232$, $df=10$, $p<0.05$) augmentation of quinine-sensitive monoamine currents observed in mouse astrocytes after cocaine chronic treatment suggests that glial monoamine transporters were activated following chronic cocaine treatment.

Discussion

DA has been shown to play important roles in the development of locomotor sensitization to repeated cocaine administration. It is known that glutamate- and GABA-mediated neuronal circuits control locomotor activation after cocaine administration, and there are important changes in these mediator's levels related to locomotor sensitization (Torregrossa and Kalivas, 2008). However, DA is still the mediator that triggers these changes; that encodes information about the drug reward and the associated drug experience (Torregrossa and Kalivas, 2008). Also, brain regions containing DA neuronal terminals are thought to mediate the persistence, or expression, of sensitization (Kalivas and Stewart 1991). DA levels in striatum and nucleus accumbens and DA clearance are important factors mediating cocaine's pleasurable effects (Volkow and Li, 2005). DAT, and NET (norepinephrine) transporters from the SCL6 transporter family tightly regulate extracellular DA levels (Gether et al, 2006). Repeated cocaine administration induces long-lasting changes in striatal DAT activity (Zahniser et al., 1995; Torregrossa and Kalivas, 2008). Some authors report increased striatal binding of specific DAT blockers after cocaine treatment, suggesting the amount of DAT expression is augmented (Little et al., 1993; Staley et al., 1994). However, several other groups have observed selective decreases in DAT density in the nucleus accumbens 10–60 days after stopping repeated cocaine administration (Kuhar and Pilotte, 1996). This discrepancy can be explained by cocaine's induction of DAT trafficking to the external membrane, while there are significant reductions of the DAT fraction in cytoplasm, and in overall DAT proteins after cocaine treatment (Little et al., 2002).

This becomes even more complicated when one takes into consideration the regional striatal differences in DAT concentrations and the differences between primate and murine species. Unlike in rats and mice, cocaine-activated DA levels in primates are augmented in ventromedial striatum, while in the dorsal and central striatum cocaine actually reduces basal DA concentrations (Bradberry et al., 2000). Because the dorsal striatum is implicated in motor activity through supplementary motor areas, cocaine probably has different effects on motor activation in primates as compared to rats and mice. Upregulation of DA uptake in dorsal striatum has been reported in fatal cocaine overdose victims (Mash et al., 1997). In addition, an unexpected 3–5-fold increase in DA clearance in dorsal striatum of rats has been observed after chronic treatment with DAT inhibitors (Ng et al., 1992; Zahniser et al., 1999). The kinetic activation of DAT by high levels of DA is not sufficient to explain the ability of systemically administered DAT inhibitors to anomalously increase DA clearance (Zahniser et al., 1999). Mice, genetically expressing markedly reduced striatal DAT, exhibit naturally increased locomotor activity, DA clearance and cocaine responsiveness, similar to that of “sensitized” normal mice (Rao et al., 2013).

Increased DA clearance in the face of reduced DAT after chronic cocaine suggests other compensatory mechanisms are playing a role in clearing DA. A parsimonious way to complete the “puzzle” of DA uptake changes after chronic cocaine injections is to suggest the recruitment of additional and different DA transporters (Zahniser et al., 1995; Kuhar and Pilotte, 1996; Little et al., 2002). These transporters must have a higher DA capacity than DAT to explain anomalously increased DA clearance after chronic cocaine treatment. We showed (Iniouchine et al., 2008) that at elevated concentrations of DA, DA uptake depends

mainly on low-affinity high-capacity transporters and was not affected by acute cocaine making this a likely mechanism.

To assess the importance of low-affinity high-capacity dopamine transporters and their participation in cocaine's locomotor behavioral sensitization we blocked these transporters using quinine and evaluated the effects. It was shown previously that quinine is a blocker of the low-affinity monoamine transporters OCT, PMAT and MATE (Bush et al., 1998; Arndt et al., 2001; Engel et al., 2004; Hiasa et al., 2006), and it effectively reduces low-affinity DA uptake in slices (Iniouchine et al., 2008). Also, quinine can substantially pass through the brain barrier with the ratio of cerebrospinal fluid to free (unbound) plasma quinine 0.55 ± 0.33 (Silamut et al., 1985). On the other hand, at high concentrations ($>100\mu\text{M}$) quinine blocks potassium channels, including the K_v -type important for muscle relaxation. It has been suggested that blockade of these potassium channels can cause cardiac arrest during contraction and is the main cause of "drug overdose" for addicts that used heroin mixed with quinine (Greene et al., 1974). Quinine exhibits both excitatory and inhibitory effects on the gross behavior of mice; these biphasic effects were dose-related, with inhibition at higher doses (5–15 mg/kg) that substantially interact with dopaminergic systems (Wambebe et al., 1989). This supports our findings that quinine alone at 10 mg/kg has mild behavioral inhibition on mice during our experiments.

After repeated injections of cocaine + quinine (15 mg/kg cocaine and 10 mg/kg quinine) over 10 days in mice, effects of cocaine + quinine co-administration became more pronounced with prolonged use of the mixture, most prominently beginning the second 5 days of co-administration. The time course of development of locomotor behavioral sensitization in cocaine alone and cocaine + quinine treated animals were similar, and coincided with the known time course for augmented DA levels after cocaine injections (Kalives and Duffy, 1993; Zahniser et al., 1999; Nelson et al., 2009), suggesting that cocaine determines the development of the effect. Quinine only affected the amplitude, or degree of sensitization, and only after 5 days of daily injections, suggesting the DA levels may also be augmented in cocaine + quinine treated animals. Could cocaine (or the everyday flow of elevated DA elicited by cocaine) have contributed to the over-expression of low-affinity DA transporters? To study this possibility we measured transporter-associated currents produced by the application of a low-affinity transporters substrate, ASP+.

The concentration of ASP+ used in these experiments, $10\mu\text{M}$, was high enough to ensure that ASP+-transport will be mediated mainly via low-affinity high-capacity transporters (Iniouchine et al., 2008). Additionally, we verified the sensitivity of ASP+-elicited currents to quinine. We demonstrated previously that ASP+ is accumulated selectively in glia, but not in neurons (Inyushin et al., 2012; 2013), suggesting that low-affinity DA transporters are mainly situated in astrocytes. Therefore, we measured ASP+-elicited currents in astrocytes in brain slices from VTA. Transporter-associated quinine-sensitive currents were significantly enhanced in astrocytes from cocaine-treated mice suggesting there is an over-expression of low-affinity transporter uptake in cocaine-treated animals.

As mentioned earlier, some authors report increased striatal binding of specific DAT blockers after cocaine treatment, suggesting the amount of expressed DAT is augmented

(Little et al., 1993; Staley et al., 1994). However, there is no proof it was actually neuronal DAT involved, and there could be glial norepinephrine transporter NET involved as well. The NET transporter was found in cortical astrocytes (Inazu et al., 1999; Takeda et al., 2002). It was shown that NET participates in DA uptake (Horn, 1973) and, indeed, it has a greater affinity for DA than does the DAT itself (Gu et al., 1994). It was shown directly using specific and nonspecific monoamine transporter inhibitors that DA uptake in caudate and nucleus accumbens depends primarily on the DAT, but DA uptake in frontal cortex depends primarily on the NET (70% versus 20%) (Moron et al., 2002). In rat cortical astrocytes at low concentration (100 nM), the Na⁺-dependent DA uptake appears to occur mainly via the NET (80% versus 20% with low-affinity transporters) (Takeda et al., 2002). But at high concentrations of DA, the uptake mainly involves low-affinity quinine-sensitive transporters (Iniouchine et al., 2008). In our experiments reported here, transporter-associated currents were quinine sensitive, suggesting quinine-sensitive transporters were involved.

In summary, the locomotor behavioral sensitization to repeated cocaine injections was enhanced by co-administration of quinine. Additionally, the activity of low-affinity DA transporters was significantly enhanced in astrocytes of cocaine-treated animals, showing their role is increasing after the treatment. These data allow us to suggest the involvement of low-affinity DA uptake transporters, such as OCT mainly concentrated in astrocytes, in the development of cocaine behavioral effects. Since these transporters could be playing a major role in regulating brain DA concentrations after chronic abuse of a variety of CNS stimulant drugs, it is likely that the contribution of astrocytes to the addiction, tolerance/sensitization process be further considered. The well-known 5-HT₃ antagonists tropisetron and ondansetron are good substrates for OCT (Tzvetkov et al., 2012). Ondansetron, given after either acute or chronic withdrawal from repeated cocaine-sensitization dosing regimens, reverses the expression of sensitization and inhibits self-administration (Davidson et al., 2002). While this action was attributed to receptor blockade, the mechanism could also be interpreted as an OCT-mediated effect.

Acknowledgments

This research was supported by G12 MD007583 to Mikhail Inyushin, Legier Rojas, Gerónimo Maldonado-Martínez and Priscila Sanabria; SC2 GM095410 to Yuri Kucheryavkh, SC1 GM088019 to Misty Eaton and R01 NS065201 to Serguei Skatchkov.

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Highlights

- We hypothesized that glial low-affinity transporters participate in DA clearance.
- Quinine-sensitive glial transporter current became enhanced after chronic cocaine.
- Quinine also enhanced cocaine-induced locomotor sensitization.
- Quinine block of glial DA transport may cause enhanced cocaine behavioral effects.

Figure 1.

The graph shows the mean $\pm$ S.E.M. of the group average distance moved (cm) during the first 30 min after injections, in two experimental and control groups, over a course of 10 days of treatment. After habituation to the behavioral testing enclosures, mice were injected i.p daily, 5 days per week for 10 days with cocaine (15 mg/kg, closed squares), or cocaine + quinine (15 mg/kg + 10 mg/kg, closed circles) or just with quinine (10 mg/kg, open circles) as a control. The distance each mouse moved was recorded each minute. Asterisks denote a statistically significant change in total activity (* $p < 0.0001$) between “Cocaine” and “Cocaine+Quinine” treated groups.

Figure 2.

Time course of effects for cocaine, cocaine + quinine or control (quinine only) groups. Data shown are the group averaged distance moved (cm) over 120 minutes following injections (recorded each minute) compared on days 1, 2 and 3 and days 8, 9 and 10. It can be seen that the cocaine and the cocaine + quinine effects grow substantially with subsequent injection, with the increased sensitization effect being sustained for approximately 30 min. There was little difference in the time scale of behavioral activation between the cocaine and cocaine + quinine groups during the first three days, while the magnitude of the effect was enhanced in cocaine + quinine treated animals during the last days (N=4–6 in each group).

Figure 3.

Transporter-associated currents in mouse astrocytes from VTA. Panel A shows the ASP⁺-transporter-induced current in a representative control mouse astrocyte. Panel B shows the transporter currents in a representative astrocyte from a cocaine-treated mouse. ASP⁺ alone (10 μ M) or ASP⁺ (10 μ M) plus Quinine (100 μ M; ASP+Q) were applied. Ba²⁺ (100 μ M) was added to the ACSF to reduce interference from potassium currents.

Table 1

Table shows the results for oneway ANOVA; for days 3, 6, 7, 8, 9, 10 there was a statistical difference between means ($p < 0.05$), most importantly between means in **cocaine only** and **cocaine+quinine** groups. In all post hoc test statistical significance was seen only between control and cocaine only/control and cocaine + quinine; $p < 0.001$.

Day	Group $\bar{x}$	F	p^*
D3	control $\bar{x} = 77.16$ cocaine only $\bar{x} = 391.82$ cocaine and quinine $\bar{x} = 318.08$	6.527	<0.05
D6	control $\bar{x} = 99.36$ cocaine only $\bar{x} = 469.38$ cocaine and quinine $\bar{x} = 518.15$	12.623	<0.05
D7	control $\bar{x} = 94.24$ cocaine only $\bar{x} = 494.81$ cocaine and quinine $\bar{x} = 388.13$	7.812	<0.05
D8	control $\bar{x} = 91.43$ cocaine only $\bar{x} = 400.11$ cocaine and quinine $\bar{x} = 453.19$	4.453	<0.05
D9	control $\bar{x} = 97.38$ cocaine only $\bar{x} = 409.24$ cocaine and quinine $\bar{x} = 537.14$	5.536	<0.05
D10	control $\bar{x} = 97.00$ cocaine only $\bar{x} = 412.23$ cocaine and quinine $\bar{x} = 555.78$	4.877	<0.05

* Bonferroni post hoc correction $p < 0.05$ within all groups