Strain Differences in Basal and Cocaine-Evoked Dopamine Dynamics in Mouse Striatum

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ABSTRACT

In vivo microdialysis was used to characterize basal dopamine (DA) dynamics and cocaine-evoked DA levels in the striatum of 129/Sv-ter, C57BL/6J, DBA/2J, and Swiss-Webster mice. Basal dialysate levels of DA did not differ in the four strains tested. Similarly, the no net flux method of quantitative microdialysis revealed no difference in extracellular levels between strains. However, the in vivo extraction fraction of DA was significantly less in 129/Sv-ter (53%) mice compared with C57BL/6J (68%), DBA/2J (69%), and Swiss-Webster (67%) mice, indicating a lower rate of basal DA uptake in the 129/Sv-ter strain. Perfusion of K⁺ (60 and 100 mM) through the microdialysis probe significantly increased dialysate DA levels and there was no difference between strains in the magnitude of this effect. The acute administration of cocaine (5–20 mg/kg i.p.) increased DA levels in the four strains tested. Cocaine-evoked DA levels (in nanomoles) were significantly greater in 129/Sv-ter compared with C57BL/6J, DBA/2J, or Swiss-Webster mice after administration of either 5, 10, or 20 mg/kg cocaine. However, the percentage increase in DA did not differ across strains. These data demonstrate that there are strain-related differences in basal DA dynamics in the striatum of the mouse. Basal DA uptake was reduced in striatum of 129/Sv-ter mice compared with C57BL/6J, DBA/2J, or Swiss-Webster mice. In addition, the response of DA levels to cocaine may be enhanced in 129/Sv-ter compared with C57BL/6J, DBA/2J, or Swiss-Webster mice.

Recent advances in homologous recombination and gene knockout strategies have permitted the development of mouse strains that overexpress or lack specific genes normally expressed in the mammalian central nervous system (Brodkin et al., 1998; Kelly et al., 1998; Jinnah et al., 1999). These mutant strains provide an important tool for identifying the genetic basis of complex behaviors and the role of specific neurotransmitter systems in mediating a particular phenotype. However, because the phenotype resulting from such genetic manipulations can reflect alterations in the targeted gene as well as interactions with other background genes, characterization of the behavioral and neurochemical phenotype of the strain in which a mutation will be evaluated is essential for identifying the effect of a particular genetic manipulation (Bosy and Ruth, 1989; Castellano et al., 1996; Crawley et al., 1997). This information also enables the selection of parental strains and breeding techniques most appropriate for expression of the phenotype of a targeted mutation (Gerlai, 1996; Crawley et al., 1997; Philips, 1997; Puglisi-Allegra and Cabib, 1997).

Embryonic stem cells from substrains of 129/Sv mice are most commonly used in targeting experiments (Sedivy and Joyner, 1992). C57BL/6J mice are typically used for breeding and as the background strain for spontaneous mutations (Erwin et al., 1993; Brodkin et al., 1998). Pharmacogenetic studies have shown that the spontaneous behavior of these and other commonly used inbred strains of mice differ (Bosy and Ruth, 1989; Erwin et al., 1993; Wasserman and DePamphilis, 1993; Grahame and Cunningham, 1995; Tolliver and Carney, 1995; Imperato et al., 1996; Tecott et al., 1996; Crawley et al., 1997; Miner, 1997). Strain-related differences in sensitivity to the behavioral effects of cocaine and other drugs of abuse also have been documented (Sanghera et al., 1990; Erwin et al., 1993; Grahame and Cunningham, 1995; Castellano et al., 1996; Minner, 1997; Rocha et al., 1997; Sallinen et al., 1998). Although a systematic examination of the behavioral phenotypes of inbred mouse strains has begun, data regarding the relationship between genetic background and brain neurochemistry are limited (Erwin et al., 1993; Womer et al., 1994; Richter et al., 1995; Brodkin et al., 1998; Zocchi et al., 1998). The present studies were conducted to address this issue.

The role of dopamine (DA) neurons comprising the mesostriatal and mesolimbic systems in the mediation of spontaneous and drug-evoked locomotor activity is well documented (Morse et al., 1995; Miner, 1997; Cabib et al., 1998). An involvement of DA neurons in mediating the reinforcing effects of cocaine and other drug of abuse also has been postu-
lated (Morse et al., 1995; Miner, 1997; Philips, 1997). Fundamental questions, however, exist as to whether differences, among inbred mice strains, in spontaneous or drug-evoked activity also are associated with differences in basal DA dynamics. It is also unclear as to whether the responsiveness of DA neurons to cocaine differs across genotype. Accordingly, the present studies used the no net flux method of quantitative microdialysis (Justice, 1993; Parsons and Justice, 1994) to characterize basal DA dynamics in the striatum of three inbred mouse strains (C57BL/6J, DBA/2J, 129/Sv-ter) commonly used in neuroscience research (Erwin et al., 1993; Grahame and Cunningham, 1995; Crawley et al., 1997; Miner, 1997). Conventional dialysis was used to characterize K⁺ and cocaine-evoked DA levels in these same strains. These data also were compared with those obtained in outbred Swiss-Webster mice.

**Materials and Methods**

**Animals**

Adult male C57BL/6J and DBA/2J (Jackson Laboratories, Bar Harbor, ME), 129/Sv-ter (obtained from Dr. Renee Hen, Columbia University, New York, NY), and Swiss-Webster mice (Taconic Farms, Germantown, NY) were housed five per cage in a temperature-controlled colony room. Mice were age-matched and weighed 25 to 48 g at the commencement of experiments. They were maintained on a 12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM) with laboratory chow and water available ad libitum. The animal facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care and all experiments were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Division of Intramural Research/National Institute on Drug Abuse/National Institutes of Health. Animals were habituated to the colony for at least 2 weeks before the commencement of experiments.

**Stereotaxic Surgery and Probe Implantation**

Mice were anesthetized with ketamine (80 mg/kg i.p.) and xylazine (8 mg/kg i.p.) and placed in a stereotaxic apparatus equipped with a mouse adapter (David Kopf, Topanga, CA). A microdialysis guide cannula (CMA 11; CMA Inc., Nagog, MA) was inserted into the dorsal striatum according to the atlas of Slotnick and Leonard (Slotnick and Leonard, 1975). The stereotaxic coordinates were as follows: AP, 0.5 mm; L, 2.1 mm; and V, 2.1 mm.

Four days after cannula implantation, a microdialysis probe (2.0-mm membrane, 6000 mol. wt. cutoff; CMA 11) was inserted into the guide cannula. The animals were placed in a Plexiglas test chamber (17 × 19 × 19.5 cm) and the inlet tubing of the probe was connected to a microinfusion pump via a single-channel quartz-lined flow rate of 0.3 μl/min.

**Microdialysis Procedure**

**Basal DA Dynamics.** The no net flux method of quantitative microdialysis (Lonnroth et al., 1987; Bungay et al., 1990; Justice, 1993; Parsons and Justice, 1994) was used to assess basal DA dynamics in the four mouse strains tested. On the day of experiments, the flow rate of the probe was increased to 0.6 μl/min. Perfusate samples were collected after a 120-min equilibration period. Five concentrations of DA (Cᵢₙ, 0, 2, 10, 20, and 30 nM) were added to the perfusate, in random order, and the amount of DA gained to or lost from the dialysis probe was determined at each Cᵢₙ concentration.

**K⁺-Stimulation.** After a 120-min equilibration period, three consecutive 30-min dialysate samples were collected for determination of basal dialysate DA levels. The aCSF was then replaced with that containing 60 mM KCl and an additional sample collected. The K⁺-containing aCSF was then replaced with physiological aCSF and two consecutive samples were again collected. This sequence was then repeated with 100 mM KCl. When the K⁺ concentration in the perfusate was increased, the concentration of Na⁺ was decreased accordingly to maintain osmolality.

**Cocaine-Evoked DA Levels.** Basal dialysate DA levels were determined as described above. Animals received an i.p. injection of either saline or 5 mg/kg cocaine and three consecutive 30-min dialysate samples were obtained. Animals then received i.p. injections of 10 and 20 mg/kg cocaine. Three 30-min dialysate samples were collected after each injection.

**Quantification of DA Levels.** The concentration of DA in dialysate samples was quantified with HPLC coupled to electrochemical detection. Chromatographic separations were performed with an HPLC column (i.d.: 2 (100 mm) packed with octadecylsilane (C18) on microparticulate (3 μM silica gel (SepStik; BAS, West Lafayette, IN) and a dual piston pump (PM 80; BAS)). The mobile phase consisted of 150 mM Na₂HPO₄, 1 mM EDTA, 1.5 mM 1-octanesulfonic acid, and 13% (v/v) MeOH, and an apparent pH of 5.0. The flow rate of the mobile phase was 500 μl/min. Electrochemical detection was accomplished with a BAS LC-4C amperometric detector. The applied potential was 0.700 V versus Ag/AgCl. Output from the detector was recorded on a dual pen chart recorder. Standard curves (0–40 nM) were used to quantify DA levels in dialysate samples. The detection limit for DA was 1.0 nM.

**Histology**

After the completion of experiments, animals were sacrificed by decapitation and the brains removed. Thirty-micrometer frozen sections were cut in a cryostat for histological verification of cannula placements.

**Data Analysis**

Only data from animals with probe placements confined to the dorsal striatum were used for data analysis. Basal extracellular DA concentrations and the probe extraction fraction were estimated with the no net flux method of quantitative microdialysis (Bungay et al., 1990; Justice, 1993). The amount of DA gained to or lost from the probe during dialysis (Cᵢₙ – Cᵥ) was plotted as a function of the Cᵢₙ. The Cᵢₙ value at which there is no net flux of DA across the dialysis membrane corresponds to equilibrium conditions and provides an unbiased estimate of DA concentration in the extracellular fluid surrounding the dialysis probe (Bungay et al., 1990; Justice, 1993; Parsons and Justice, 1994). The slope of the regression line is equal to the extraction fraction and, for DA, has been shown to provide an index of DA uptake (Parsons and Justice, 1994). Thus, pharmacological treatments that increase DA uptake increase the probe extraction fraction, whereas pharmacological treatments that decrease DA uptake decrease extraction fraction (Bungay et al., 1990; Justice, 1993; Parsons and Justice, 1994). One-way ANOVAs were used to compare extracellular DA concentration and extraction fraction across genotype. The effects of the K⁺ and cocaine challenges on dialysate DA levels were analyzed with three-way (genotype × challenge × time) or two-way (genotype × time) ANOVA with repeated measures over time. Simple effects tests or pairwise group comparisons were used to probe significant differences identified by ANOVA. The Greenhouse-Geisser correction for multiple time measures was used when appropriate (Zar, 1974). The accepted level of significance for all tests was P ≤ .05.
TABLE 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Count</th>
<th>Mean ± S.E.</th>
<th>95% CI Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>SW</td>
<td>22</td>
<td>3.7 ± 0.2</td>
<td>3.1–4.2</td>
</tr>
<tr>
<td>DBA/2J</td>
<td>19</td>
<td>4.3 ± 0.4</td>
<td>3.5–5.1</td>
</tr>
<tr>
<td>C57BL/6J</td>
<td>19</td>
<td>4.3 ± 0.3</td>
<td>3.6–5.0</td>
</tr>
<tr>
<td>129/Sv-ter</td>
<td>27</td>
<td>4.7 ± 0.3</td>
<td>4.0–5.4</td>
</tr>
</tbody>
</table>

Results

Dialysate DA Levels and Basal DA Dynamics. Table 1 shows basal dialysate concentrations of DA in C57BL/6J, DBA/2J, 129/Sv-ter, and Swiss-Webster mice obtained via conventional dialysis techniques. ANOVA showed no difference between strains in DA concentrations (\(F_{3,63} = 1.87, P = .14\)). The zero point on the y-axis represents steady-state conditions when there is no net flux of DA across the dialysis membrane and corresponds to the extracellular concentration of DA. No difference between strains in extracellular DA concentrations was observed (\(F_{3,63} = 1.87, P = .14\)). DA concentrations were 7.7 ± 1.2, 6.7 ± 0.8, 9.8 ± 1.3, and 7.1 ± 0.7 nM in C57BL/6J, DBA/2J, 129/Sv-ter, and Swiss-Webster, respectively.

The dialysate concentrations at zero added perfusate DA were 4.6 ± 0.4, 4.4 ± 0.5, 4.6 ± 0.5, and 4.4 ± 0.4 nM in C57BL/6J, DBA/2J, 129/Sv-ter, and Swiss-Webster, respectively, and correspond to the dialysate concentrations obtained with conventional microdialysis techniques. The slope of the regression lines shown in Fig. 1 gives the extraction fraction of DA. As shown in Fig. 1, there were significant strain-related differences in extraction fraction (\(F_{3,63} = 4.19, P = .0082\)). Post hoc analysis revealed that the extraction fraction of the 129/Sv-ter strain (53%) was significantly less than that of the C57BL/6J (68%), DBA/2J (69%), and Swiss-Webster (67%) strain (Tukey’s honestly significant difference test, \(P = .05\)).

Effect of K⁺-Stimulation on Dialysate DA Levels. Perfusion of KCl through the dialysis probe significantly increased dialysate levels of DA and the magnitude of this effect varied as a function of time (Fig. 2). Analysis of absolute DA levels (nanomolar) revealed no differences in K⁺-evoked DA levels between strains (Fig. 2A) (time: \(F_{3,216} = 34.9, P = .001\); strain: \(F_{3,36} = 2.03, P = .1\); strain × time interaction: \(F_{18,216} = 0.78, P = .6\)). There were also no significant differences in K⁺-evoked DA levels when the data were expressed as a baseline of absolute DA levels (time: \(F_{3,288} = 34.3, P < .001\); strain: \(F_{3,36} = 0.12, P = .9\); strain × time interaction: \(F_{24,288} = 0.41, P = .9\)). The mean peak percentage increase in DA in the four strains tested ranged from 232 to 378% of basal levels after perfusion of 60 mM KCl and from 713 to 1124% after perfusion of 100 mM KCl (Fig. 2B).

Effect of Cocaine on Dialysate DA Levels. Injections of saline did not modify dialysate levels of DA in any of the four strains tested (data not shown). The administration of cocaine significantly increased DA levels in all strains and at each of the doses tested. This increase was apparent when DA levels were expressed as actual dialysate concentrations or as a percentage of basal values (Fig. 3, A and B). Analysis of dialysate levels (nanomolar) revealed a significant difference between strains in the response to cocaine (\(F_{3,42} = 4.2, P = .01\)). DA levels were significantly greater in 129/Sv-ter mice compared with C57BL/6J, DBA/2J, or Swiss-Webster.
mice, respectively, in the first 30 min after administration of 5, 10, or 20 mg/kg cocaine. The response of 129/Sv-ter mice was also significantly greater than that of Swiss-Webster mice 60 min after administration of 5 mg/kg cocaine. It was also significantly greater than that of C57BL/6J and Swiss-Webster mice 60 and 90 min after the administration of 10 mg/kg cocaine. In contrast, when the data were expressed as a percentage of basal levels, no significant difference between strains in the response to cocaine was seen (time: $F_{9,378} = 19.54, P < .001$; strain: $F_{3,42} = .76, P = .52$; strain $\times$ time interaction: $F_{27,378} = .56, P = 1.0$).

**Histology.** Fig. 4 illustrates the location of microdialysis probes used in this study. Only animals with probe placements in the dorsal striatum and that did not pass through the ventricles were used for data analyses.

**Discussion**

These data demonstrate genotype-dependent differences in basal DA dynamics and cocaine-evoked DA levels in the striatum of the mouse. The in vivo extraction fraction of DA is significantly less in 129/Sv-ter compared with C57BL/6J, DBA/2J, and Swiss-Webster mice. The acute administration of cocaine increased dialysate DA levels in all strains. Analysis of the percentage increase in DA levels produced by graded doses of cocaine indicated no differences between strains in this effect. However, analysis of absolute DA levels revealed a significantly greater increase in cocaine-evoked DA levels in the 129/Sv-ter strain compared with the C57BL/6J, DBA/2J, or Swiss-Webster strains.

In vivo microdialysis has been used extensively to characterize basal and drug-evoked neurotransmitter levels in discrete regions of the central nervous system (for review, see Thompson and Shippenberg, 1997). In recent years, however, it has become apparent that although conventional microdialysis techniques provide information regarding the relative concentration of a neurotransmitter in the extracellular space, they do not permit the measurement of actual extracellular concentrations (Bungay et al., 1990; Justice, 1993; Parsons and Justice, 1994). Thus, several studies have
shown that pharmacological treatments that alter the rate of DA uptake by the DA transporter can modify the extraction fraction of the dialysis probe in vivo leading to an under- or overestimation of true extracellular concentrations (Parsons et al., 1991a; Cosford et al., 1996; Sam and Justice, 1996). Pharmacological manipulations that increase DA reuptake increase extraction fraction, whereas manipulations that decrease uptake decrease this parameter. Therefore, increases in dialysate DA levels may reflect increases in extracellular concentration that occur as a consequence of increased release and/or decreases in uptake (for review, see Thompson and Shippenberg, 1997). Similarly, pharmacological or genetic manipulations that increase DA release and uptake may result in no alteration in extracellular DA levels. Due, however, to the increase in DA uptake and corresponding increase in probe extraction fraction, an increase in dialysate levels may be seen.

In the present study, the no net flux method of quantitative microdialysis (Justice, 1993; Parsons and Justice, 1994) was used to simultaneously assess basal extracellular DA concentrations and extraction fraction in the four mouse strains tested. Dialysis levels obtained in conventional microdialysis techniques also were compared with that from no net flux experiments. Conventional and no net flux experiments yielded similar basal dialysate DA levels. Regardless of the method used, no differences between strains in basal DA levels were seen. Analogous to dialysate DA levels, basal extracellular concentrations of DA did not differ between mouse strains. Although extracellular DA levels appeared higher in the 129/Sv-ter strain, this effect was not statistically significant. No net flux experiments, however, revealed that the extraction fraction for DA is significantly less in 129/Sv-ter mice compared with C57BL/6J, DBA/2J, or Swiss-Webster mice. In contrast to DA uptake, pharmacological manipulations that modify the release or metabolism of DA do not affect the in vivo extraction fraction of DA. Therefore, the finding of a decrease in extraction fraction in 129/Sv-ter mice is evidential that the basal rate of DA uptake is decreased in this mouse strain. Furthermore, the fact that extracellular DA levels do not differ in the 129/Sv-ter, C57BL/6J, DBA/2J, and Swiss-Webster strains suggests that basal DA uptake and release are reduced in 129/Sv-ter mice. Voltammetry studies assessing DA release and the kinetics of DA transport in these strains are currently in progress.

No differences in extraction fraction were observed between C57BL/6J, DBA/2J, and Swiss-Webster mice, indicating that basal rates of DA uptake are similar in these mouse strains. A direct assessment of DA uptake in these strains is lacking. Equilibrium binding studies, however, revealed no differences in the affinity or binding capacity of the DA transporter in striatum of C57BL/6J and DBA/2J mice (Erwin et al., 1993; Womer et al., 1994). Similarly, no differences in $K_i$ values for DA binding to the transporter were observed, suggesting that the function of the DA transporter does not differ in these strains.

Cocaine binds to the DA transporter and inhibits the reuptake of DA, thereby, increasing synaptic concentrations of DA (Morse et al., 1995). In agreement with previous studies in the rodent, the acute administration of cocaine increased

**Fig. 4.** Representation of dialysis probe placements. The sampling area for probes were determined in histological sections and is represented by the area within the dotted lines.
dialysate concentrations of DA in striatum (Hadfield, 1995; Kankaanpaa et al., 1996; Martin-Fardon et al., 1996). A significant increase was observed in all strains after administration of cocaine (5–20 mg/kg). The response to cocaine did not differ significantly in C57BL/6J, DBA/2J, or Swiss-Webster strains. Thus, regardless of whether the data were expressed as absolute levels (nanomolar) or as a percentage of basal values, no difference in cocaine (5–20 mg/kg)-evoked DA levels were seen. Data are consistent with in vivo data regarding the inhibition of [3H]DA uptake by cocaine in striatal synaptosomes of DBA/2J and C57BL/6J strains (Bosy and Ruth, 1989). Collectively, they indicate that the response of striatal DA neurons to cocaine does not differ in these mouse strains. Interestingly, amphetamine-evoked DA levels are greater in the nucleus accumbens of C57BL/6 compared with DBA/2J mice (Zocchi et al., 1998). The different results obtained in these studies may indicate strain differences in the response to amphetamine compared with cocaine. Alternatively, the responsiveness of mesostraital and mesoaccumbens DA neurons to these psychostimulants may differ.

No strain differences in the response to cocaine were observed when cocaine-evoked DA level data were expressed as a percentage of basal values. These results are especially surprising because of the decreased basal DA uptake and increased release observed in 129/Sv-tet mice, and they suggest that strain differences in the basal activity of DA neurons can occur in the absence of an altered response to cocaine. Interestingly, however, analysis of absolute DA levels revealed a significantly greater effect of cocaine (5 mg/kg) in 129/Sv-tet compared with either C57BL/6J, DBA/2J, or Swiss-Webster mice. An enhanced response of 129/Sv-tet animals, relative to that of either C57BL/6J, DBA/2J, or Swiss-Webster strains was also apparent after administration of 10 and 20 mg/kg cocaine.

At present, it remains unclear whether the expression of a behavioral response to a drug or sensitivity to a particular drug effect is dependent on the change, relative to basal values, in neurotransmitter concentration or exceeding a set-point concentration. If, however, the latter is the case, then the present results would predict an enhanced response of 129/SvJ mice to those behavioral effects of cocaine that are mediated by mesostriatal neurons. In this regard, it is important to note that behavioral studies have shown that C57BL/6J and 129/SvJ mice do not differ in sensitivity to the psychomotor stimulant effect of cocaine (Miner, 1997). Whether, however, cocaine-evoked stereotypy, an effect attributed to an increase in mesostriatal DA (Kelly et al., 1975), rather than to increases in mesoaccumbal DA levels (Kelly and Iversen, 1976), is greater in 129/SvJ mice has not been evaluated.

In summary, the results of conventional and no net flux microdialysis experiments demonstrate gene-dependent differences in DA dynamics in mouse striatum. The basal uptake of DA in the 129/Sv-tet substrain of 129SvJ/mice is reduced relative to C57BL/6J, DBA/2J, and Swiss-Webster mice, whereas the response of striatal DA neurons to cocaine may be enhanced relative to C57BL/6J, DBA/2J, and Swiss-Webster strains. These data add to a growing body of evidence indicating genetic differences in mesostriatal DA neurochemistry. In addition, they suggest that the 129/Sv-tet substrain may be a particularly interesting genetic background for studies assessing DA transporter function.

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References


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