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European Journal of Pharmacology 319 (1997) 31–36

ejp

Short communication

Extracellular aspartate concentration increases in nucleus accumbens after cocaine sensitization

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Received 19 November 1996; accepted 22 November 1996

Abstract

Rats were sensitized to cocaine (15 mg/kg, i.p.) by 6 daily injections followed by a 48 h withdrawal prior to cocaine challenge. Involvement of excitatory amino acids in behavioral sensitization was assessed by comparing extracellular levels of aspartate and glutamate in the core of the nucleus accumbens in response to the first cocaine injection and the final cocaine challenge. Intracerebral microdialysis of the nucleus accumbens in freely moving awake rats allowed the comparison of behavioral state with extracellular aspartate and glutamate concentrations. Increased nucleus accumbens extracellular concentration of aspartate, but not glutamate, was observed in rats exhibiting behavioral sensitization to cocaine.

Keywords: Aspartate; Cocaine; Glutamate; Nucleus accumbens; (Rat); Sensitization

1. Introduction

Recently, the importance of excitatory amino acids such as aspartate and glutamate in the behavioral effects of cocaine has been under investigation. Intra-accumbens injection of the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovaleric acid (APV) blocks cocaine and amphetamine, but not caffeine, stimulated locomotion (Pulvirenti et al., 1991; Kelley and Throne, 1992). The observation that cocaine increases the extracellular concentration of aspartate and glutamate provides more direct evidence that cocaine activates neurons which release excitatory amino acids (Smith et al., 1995).

An intriguing feature of psychomotor stimulants such as cocaine is the phenomenon of behavioral sensitization. Enhancement of the behavioral response to psychomotor stimulants after repeated administration has been observed in both animals and humans. Although this phenomenon has been thought to result at least partly from augmented dopaminergic neurotransmission in the nucleus accumbens (Bradberry and Roth, 1989; Kalivas and Duffy, 1990), enhanced dopaminergic neurotransmission does not appear to be a prerequisite for behavioral sensitization (Segal and Kuczenski, 1992; Kalivas and Duffy, 1993). Neurotransmitters other than dopamine may be involved in behavioral sensitization, including aspartate and glutamate. The NMDA receptor antagonist MK-801 has been reported to block behavioral sensitization to the locomotor responses to cocaine in mice and to the stereotypic and convulsive responses to cocaine in rats (Karler et al., 1989), and a recent study reported an increase in extracellular concentrations of glutamate in rats sensitized to cocaine (Pierce et al., 1996).

Previous studies found that acute injection of cocaine produces far greater increases in the extracellular concentration of aspartate than of glutamate (Smith et al., 1995). Levels of aspartate as well as of glutamate were measured in the core of the nucleus accumbens in response to cocaine before and after sensitization. NMDA receptors in the core of the nucleus accumbens appear to mediate the stimulation of locomotor activity by cocaine (Pulvirenti et al., 1994), and this is the region in which increased extracellular glutamate was observed in rats sensitized to cocaine (Pierce et al., 1996).

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PII S0014-2999(96)00923-5

2. Materials and methods

2.1. Animals

Male Sprague-Dawley 'CD' rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 250–390 g were maintained on a 12-h light/dark cycle in a temperature- and humidity-controlled room and provided food (Purina Lab Chow) and water ad libitum. Following a 7-day acclimation period in our animal facilities, rats were anesthetized with Equithesin, and an intracerebral guide cannula (CMA/12, CMA/Microdialysis, Acton, MA, USA) was stereotaxically implanted, aimed at the coordinates +1.7 mm AP, 1.6 mm L, −6.2 mm V, with respect to bregma (Paxinos and Watson, 1986). Cannula placements were confirmed at the end of the experiment by standard histological procedures.

2.2. Microdialysis procedure

One week after surgery, each rat was briefly anesthetized with methoxyflurane, and a CMA/12 concentric microdialysis probe (0.5 mm diameter × 2 mm exposed dialysis membrane; CMA/Microdialysis, Acton, MA, USA) was inserted to extend 2 mm beyond the guide cannula into the nucleus accumbens and perfused with buffer at a rate of 2.0 μl/min as described by Smith et al. (1995). After an initial equilibration time of 3–4 h, samples were collected in 10-min fractions for an additional 2 h to establish baseline values in the awake, freely moving rat. Cocaine (15 mg/kg, National Institute on Drug Abuse, Washington, DC, USA) or saline (1 ml/kg) was then injected i.p. and 10-min fractions collected for an additional 2 h. This dose of cocaine (15 mg/kg, i.p.) is insufficient to produce a significant increase in extracellular aspartate or glutamate upon acute injection (Smith et al., 1995). Microdialysis samples were collected on days 1 and 8 of the experiment. One group of rats was injected with cocaine during both microdialysis sessions; the other group with saline during both sessions. Within each group approximately half of the rats were injected once daily with cocaine (15 mg/kg, i.p.) on days 1 through 6, while the other half was injected with saline. All injections, with the exception of those given during the microdialysis procedures, were administered in the rat's home cage. No treatments were administered on day 7.

2.3. Behavioral evaluation

Each rat was videotaped during the sample collection period for later analysis of behavioral responses. Rats were observed for 30 s, midway through each 10-min collection period, and rated on a 6-point behavioral activation and stereotypy scale (Kunko et al., 1993) by an observer blind to treatment.

2.4. Measurement of aspartate and glutamate

Immediately after collection, an internal standard consisting of 1 nmol each of deuterium-labeled aspartate (L-aspartic-2,3,3-d_3 acid) and glutamate (L-glutamic-2,3,3,4,4-d_5 acid) (CDN isotopes, Montreal, Canada), dissolved in 1 M hydrochloric acid, was added to the samples, which were then stored frozen at −80°C. An external standard curve was also prepared with each tube containing internal standard as well as various amounts of unlabeled aspartate and glutamate. Samples were derivatized as previously described and injected into a Hewlett-Packard 5890A gas chromatograph coupled by a capillary direct interface to a Hewlett-Packard 5970B mass selective detector in the electron impact mode (Smith et al., 1995). The ratios of endogenous peak area to internal standard peak area were converted to concentrations of aspartate and glutamate using the standard curve.

2.5. Statistics

Areas under the curve (AUCs) were calculated for the behavioral scores and extracellular concentrations (pmol/μl) of aspartate and glutamate obtained over the first hour after injection. AUCs did not include data obtained in the second hour after injection, as few changes were noted during that time period. The AUCs of the extracellular amino acid concentrations from the 2 microdialysis procedures for each rat were compared using 2-way repeated measures analysis of variance (ANOVA). This allowed each rat to serve as its own control. The behavioral scores represented discrete random variables, hence nonparametric statistical tests were required. An overall Kruskal-Wallis analysis of the 8 AUCs (4 treatment groups, 2 microdialysis procedures) was used, followed by the Mann-Whitney *U*-test for each treatment group. For purposes of time-course data presentation, aspartate content was normalized to percent of baseline. Baseline was calculated as the average of the 3 samples immediately preceding injection. Baseline values for the extracellular amino acids were also compared using 2-way repeated measures ANOVA. A level of $P < 0.05$ was accepted as statistically significant in all comparisons.

3. Results

3.1. Behavioral sensitization to repeated cocaine injection

As predicted, rats exhibited behavioral activation following injection of cocaine (15 mg/kg, i.p.) (Fig. 1). The Kruskal-Wallis test yielded a significant treatment effect ($H = 38.917$, $P < 0.05$). Post-hoc testing with the Mann-Whitney *U*-test found that AUCs for behavioral scores were significantly increased in response to the cocaine

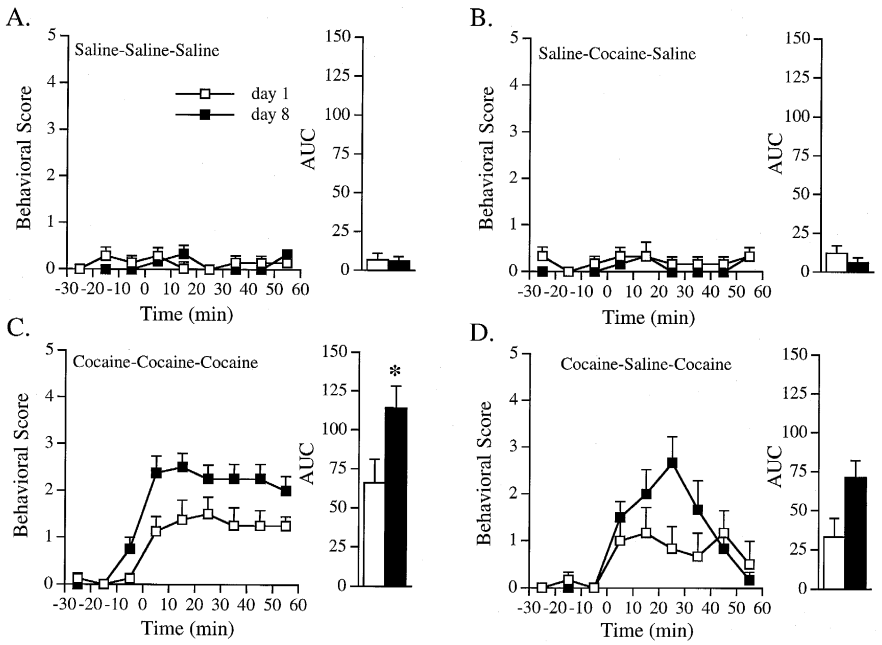

Fig. 1. Stereotypic behavior expressed during microdialysis procedures in rats injected i.p. at time 0 with either saline (panels A and B) or cocaine (15 mg/kg, panels C and D). Panel A presents results from rats ($n = 7$) which received saline injections during microdialysis procedures on days 1 and 8, as well as during the intervening 5 days prior to withdrawal. Panel B presents results from rats ($n = 6$) receiving saline injections during microdialysis procedures on days 1 and 8, as well as 6 daily injections of cocaine prior to withdrawal. Panel C represents results from rats ($n = 7$) which received cocaine injections during microdialysis procedures on days 1 and 8, as well as during the intervening 5 days prior to withdrawal. Panel D presents results from rats ($n = 6$) receiving cocaine injections during microdialysis procedures on days 1 and 8, as well as injections of saline during the intervening 5 days prior to withdrawal. Data are expressed as mean ± S.E.M. * $P < 0.05$ by Mann-Whitney U.

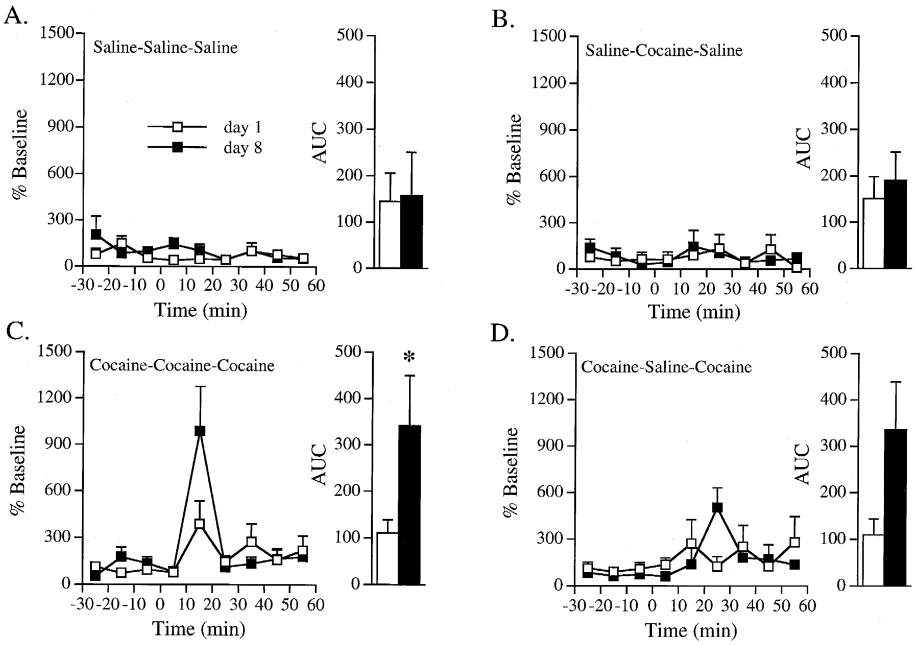

Fig. 2. The effect of repeated cocaine administration on extracellular aspartate in the nucleus accumbens, expressed as percent baseline. Baseline is the average aspartate concentration over the three 10-min collection periods preceding i.p. injection of either saline or cocaine (15 mg//kg) at time 0. Treatment groups for panels A–D are the same as in Fig. 1. Dashed line represents 100% of baseline. Baseline values in panel A are 4.3 ± 2.4 and 4.8 ± 2.7 pmol/μl for the first and second microdialysis procedures, respectively. Baseline values in panel B are 3.2 ± 1.2 and 2.4 ± 0.8 pmol/μl for the first and second microdialysis procedures, respectively. Baseline values in panel C are 1.5 ± 0.6 and 2.4 ± 0.7 pmol/μl for the first and second microdialysis procedures, respectively. Baseline values in panel D are 2.4 ± 1.1 and 3.2 ± 1.4 pmol/μl for the first and second microdialysis procedures, respectively. Histograms represent cumulative aspartate responses (AUC, area under the curve) over the first hour after injection. Data are expressed as mean ± S.E.M. * $P < 0.05$ by ANOVA and Fisher's PLSD.

challenge for rats receiving cocaine in the test chamber followed by 5 daily cocaine injections in their home cages (Fig. 1C; $Z = -2.107$, $P < 0.05$). A strong trend was observed for an increased behavioral response to cocaine in the rats which had been injected only once previously with the drug in the test chamber (Fig. 1D, $Z = -1.928$, $P = 0.0538$). Rats injected with saline in both microdialysis procedures as well as in their home cages on days 2–6, exhibited identical behavioral scores on days 1 and 8 (Fig. 1A). Furthermore, there was no change in the behavioral response to saline in rats which had received repeated cocaine injections in their home cages, but not in the test chamber (Fig. 1B).

3.2. Nucleus accumbens extracellular excitatory amino acid sensitization to repeated cocaine injection

Extracellular concentrations of aspartate in the core of the nucleus accumbens were not affected by saline injection during either the first or second microdialysis procedures, whether the rats were injected on the intervening days with saline or cocaine (Fig. 2A and 2B). On the other hand, rats which were injected with cocaine during the microdialysis procedures appeared to exhibit an enhanced response to cocaine during the second microdialysis procedure (Fig. 2C and 2D). Extracellular concentrations of aspartate were plotted in 10-min increments for the 2 microdialysis procedures and AUCs calculated over the first hour after injection of saline or cocaine. Two-way repeated measures (days 1 and 8) ANOVA revealed a significant effect of the repeated measure ($F(1,22) = 10.478$, $P < 0.05$) as well as a significant interaction of treatment and the repeated measure ($F(3,22) = 3.259$, $P < 0.05$). Rats in the treatment group receiving cocaine in the test chamber plus 5 additional daily cocaine injections exhibited a significantly larger AUC for the extracellular aspartate concentration in response to cocaine during the second microdialysis procedure as compared to that in the first microdialysis procedure (Fig. 1C, $P < 0.05$). Interestingly, rats receiving cocaine only during the microdialysis procedure, but not on the intervening days, exhibited a strong trend for an enhanced increase in extracellular aspartate during the second microdialysis procedure ($P = 0.0633$). Two-way repeated measures ANOVA detected no differences in the baseline values of aspartate among the 4 treatment groups ($F(3,22) = 0.571$, $P = 0.640$) nor between baseline values in the 2 microdialysis procedures ($F(1,22) = 0.45$, $P = 0.509$). Neither was a significant interaction of treatment and the repeated measure observed ($F(3,22) = 0.416$, $P = 0.743$).

Unlike the aspartate results, the response of extracellular glutamate was not significantly affected by repeated cocaine injection. Neither were basal levels affected. Baseline values for extracellular glutamate in the saline-injected group were 4.4 ± 1.4 and 6.6 ± 2.4 pmol/μl for the first and second microdialysis procedures, respectively. These values are similar to those previously reported in the literature (Smith et al., 1995; Pierce et al., 1996). Baseline values for the group receiving saline for the microdialysis procedures, but cocaine in their home cages, were 3.6 ± 0.6 and 3.4 ± 0.8 pmol/μl for the first and second microdialysis procedures, respectively. The rats receiving cocaine in both the test cage and their home cages had baseline glutamate values of 5.6 ± 1.4 and 5.6 ± 1.8 pmol/μl for the first and second microdialysis procedures, respectively, and the rats receiving cocaine only in the test cage had baseline glutamate values of 4.6 ± 1.6 and 4.8 ± 1.6 pmol/μl for the first and second microdialysis procedures, respectively.

4. Discussion

The extracellular concentration of aspartate, but not glutamate, was increased in the core of the nucleus accumbens in response to cocaine in behaviorally sensitized rats. Consistent with other reports in the literature, repeated injection of cocaine followed by a 48-h withdrawal period resulted in behavioral sensitization to a challenge dose of cocaine (Kalivas and Stewart, 1991; Segal and Kuczenski, 1992). In fact, there was a strong trend for sensitization in all rats injected with cocaine in the test chamber, whether they received a total of 1 or 6 cocaine injections prior to the cocaine challenge in the second microdialysis procedure. Acute injection of cocaine at the dose used in the present study (15 mg/kg) did not produce statistically significant increases in extracellular aspartate and glutamate in the nucleus accumbens. However, extracellular concentrations of aspartate were significantly increased in the behaviorally sensitized rats following the cocaine challenge in the second microdialysis procedure. No significant effects were observed on glutamate in the same sensitized rats. Although this study was not designed to distinguish the actions of cocaine in the core versus the shell of the nucleus accumbens, no increase in extracellular aspartate was detected in rats in which microdialysis probes were accidentally placed in the shell of the nucleus accumbens (data not shown). Rather than paralleling behavioral activation, the increase in extracellular aspartate is a discrete event occurring near the beginning of cocaine-induced behavioral activation. When comparing the time-course of behavioral activation with the time course of increased nucleus accumbens extracellular aspartate, it is obvious that release of aspartate into the core of the nucleus accumbens is not solely responsible for the behavioral activation occurring after cocaine challenge. Interestingly, tetrodotoxin, which reduces the extracellular aspartate response, does not block, and even appears to prolong, the behavioral activation observed after acute injection of a large dose of cocaine (Smith et al., 1995). This further argues against increased extracellular levels of aspartate being the direct causative factor for cocaine-induced behavioral activation.

The mechanism behind the increased extracellular aspartate in the sensitized rats has yet to be determined. The nucleus accumbens receives excitatory amino acid projections from the amygdala, hippocampus, and prefrontal cortex (Phillipson and Griffiths, 1985); however, no purely 'aspartatergic' pathways have been described. An earlier study found that either removal of calcium or inclusion of tetrodotoxin in the microdialysis buffer significantly reduces the magnitude of the increase in extracellular aspartate observed after a large acute dose of cocaine (Smith et al., 1995), suggesting the possibility of an impulse-driven vesicular source of the extracellular aspartate. It is possible that both aspartate and glutamate are released by cocaine, but that glutamate is more efficiently removed from the extracellular space, leaving behind relatively higher levels of aspartate. The glutamate/aspartate transporter has been demonstrated to have a greater affinity and capacity for glutamate than aspartate (Ferkany and Coyle, 1986; Klöckner et al., 1994).

The nucleus accumbens contains NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors (Cotman and Monaghan, 1987). Although glutamate can act at any of these 3 receptor types, aspartate acts preferentially at NMDA receptors (Mayer and Westbrook, 1984). Injection of the competitive NMDA receptor antagonist APV into the nucleus accumbens blocks cocaine and amphetamine stimulated locomotion (Pulvirenti et al., 1991; Kelley and Throne, 1992). The nature of excitatory amino acid involvement in behavioral sensitization is currently under discussion. Karler et al. (1989) found that systemic coadministration of the non-competitive NMDA receptor antagonist MK-801 during the induction phase prevented cocaine- and amphetamine-induced behavioral sensitization. Furthermore, it has been reported that MK-801 prevented the development of behavioral sensitization to amphetamine or cocaine at doses that do not block acute behavioral stimulation (Wolf and Jeziorski, 1993; Wolf et al., 1994). This is consistent with the finding that the time-course of the aspartate response in the present study does not parallel that of the behavioral response. One might speculate that cocaine-induced increases in aspartate levels in sensitized rats initiate the chain of events responsible for behavioral sensitization.

A recent study reports increased extracellular glutamate in rats behaviorally sensitized to cocaine (Pierce et al., 1996). However, it appears that the increase in extracellular glutamate in response to a 15 mg/kg cocaine challenge in that study partially reflects a trend for decreased baseline values of glutamate in cocaine-pretreated animals. No such decreases in baseline levels of either aspartate or glutamate were observed in the present study. In addition, Pierce et al. (1996) found an increase in the motoric response to intra-accumbens injection of AMPA in behaviorally sensitized rats, again stressing the importance of glutamatergic transmission. Initially, this report appears to be at odds with the findings in the present study, which detected no changes in extracellular glutamate in the sensitized rats. However, there are methodological differences which may explain the discrepancies between the two studies. Pierce et al. (1996) used a much higher dose of cocaine to sensitize the rats (30 mg/kg versus 15 mg/kg) and measured glutamate in rats which had been withdrawn from cocaine for 2–3 weeks. There is evidence that the mechanisms involved in behavioral sensitization following brief (1–2 days) versus long (1–3 weeks) withdrawals differ (Segal and Kuczenski, 1992; Kalivas and Duffy, 1993). Thus, the different results may reflect the fact that two different phenomena are occurring.

In summary, behavioral sensitization observed following a brief withdrawal from repeated cocaine is accompanied by an increase in extracellular aspartate in the core of the nucleus accumbens. Whether this increase represents an anticipatory event or an initiation of the enhanced behavioral response remains to be determined.

Acknowledgements

The authors gratefully acknowledge the advice of Dr. Aron Lichtman on the statistical analysis of the data. This work was supported in part by NIDA grants DA05274 and DA07027.

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