British Journal of Pharmacology (2000) 129, 582–588
www.nature.com/bjp

On the mechanism of d-amphetamine-induced changes in glutamate, ascorbic acid and uric acid release in the striatum of freely moving rats

[1]Maddalena Miele, [1]Maria A. Mura, [1]Paolo Enrico, [1]Giovanni Esposito, [1]Pier A. Serra, [1]Rossana Migheli, [1]Danilo Zangani, [1]Egidio Miele & *,[1]Maria S. Desole

[1]Department of Pharmacology, University of Sassari, Viale S. Pietro 43/B, 07100 Sassari, Italy

1 The effects of systemic, intrastriatal or intranigral administration of d-amphetamine on glutamate, aspartate, ascorbic acid (AA), uric acid, dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in dialysates from the striatum of freely-moving rats were evaluated using microdialysis.

2 d-Amphetamine (2 mg kg^{-1}) given subcutaneously (s.c.) increased DA, AA and uric acid and decreased DOPAC + HVA, glutamate and aspartate dialysate concentrations over a 3 h period after d-amphetamine. 5-HIAA concentrations were unaffected. Individual changes in glutamate and AA dialysate concentrations were negatively correlated.

3 d-Amphetamine (0.2 mM), given intrastriatally, increased DA and decreased DOPAC + HVA and aspartate dialysate concentrations, but failed to change those of glutamate, AA uric acid or 5-HIAA, over a 2 h period after d-amphetamine. Haloperidol (0.1 mM), given intrastriatally, increased aspartate concentrations without affecting those of glutamate or AA.

4 d-Amphetamine (0.2 mM), given intranigrally, increased AA and uric acid dialysate concentrations and decreased those of glutamate, aspartate and DA; DOPAC + HVA and 5-HIAA concentrations were unaffected.

5 These results suggest that d-amphetamine-induced increases in AA and uric acid and decreases in glutamate concentrations are triggered at nigral sites. The changes in aspartate levels may be evoked by at least two mechanisms: striatal (mediated by inhibitory dopaminergic receptors) and nigral (activation of amino acid carrier-mediated uptake).
British Journal of Pharmacology (2000) **129,** 582–588

Keywords: d-Amphetamine; glutamate; aspartate; ascorbic acid; uric acid; microdialysis; rat striatum

Abbreviations: AA, ascorbic acid; aCFS, artificial cerebrospinal fluid; ANOVA, analysis of variance; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; HVA, homovanillic acid; s.c., subcutaneous; XO, xanthine oxidase

Introduction

The effects of d-amphetamine on striatal dopaminergic system activity are well known: d-amphetamine increases extracellular levels of dopamine (DA) and decreases the main DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA; Zetterström *et al*., 1986; Robertson *et al*., 1991; Cadoni *et al*., 1995). DA release in response to d-amphetamine is transporter-mediated and the process has been called reverse transport (Sulzer *et al*., 1995). However, DA is not the only endogenous substance released by d-amphetamine. Extracellular ascorbic acid (AA) concentrations in the neostriatum, which range between 350 and 500 μM (Miele & Fillenz, 1996), increase by about 50–80% following systemic injection of d-amphetamine (Wilson & Wingthman, 1985; Basse-Tomusk & Rebec, 1990; Mueller & Kunko, 1990; Saponjic *et al*., 1994). Moreover, d-amphetamine increases striatal uric acid extracellular concentrations (Mueller & Kunko, 1990; Saponjic *et al*., 1994). Uric acid is the end product of ATP catabolism. The final step leads to xanthine, which is a substrate for xanthine oxidase (XO). The products of XO include uric acid and reactive oxygen species. Uric acid is an active component of the neuronal antioxidant pool; it protects AA (Sevanian *et al*., 1991) and DA (Church & Ward, 1994) from oxidation.

Apomorphine (Zetterström & Fillenz, 1988) and L-glutamate (O'Neill *et al*., 1984) raise extracellular AA levels in the rat striatum when given systemically. When given intrastriatally, apomorphine (Zetterström & Fillenz, 1988) and d-amphetamine (Wilson & Wingthman, 1985) do not raise extracellular AA, although L-glutamate causes a great increase in AA extracellular concentrations (O'Neill *et al*., 1984).

It has been shown that physiologically-induced striatal AA release is linked to impulse traffic, transmitter release and glutamate uptake (Miele *et al*., 1994). The latter finding strongly supports the proposed glutamate/AA heteroexchange model (O'Neill, 1995).

A great number of *in vitro* and *in vivo* studies have established the reciprocal dopamine-glutamate modulation of release in the basal ganglia (see Morari *et al*., 1998). In addition, an increasing body of evidence suggests that release of AA into the extracellular fluid of the brain modulates both dopaminergic (Rebec & Pierce, 1994) and glutamatergic transmission (Majewska *et al*., 1990; Rebec & Pierce, 1994). It is well established that DA is the primary mediator of d-amphetamine-induced behaviours (Robinson & Becker, 1986). The glutamatergic system is also claimed to participate in the

*Author for correspondence; E-mail: Pharmaco@ssmain.uniss.it

mediation of d-amphetamine behaviours (Desole *et al*., 1992; Karler *et al*., 1995; Ohmori *et al*., 1996), while AA administration, either systemic (Rebec *et al*., 1985; Desole *et al*., 1987) or intrastriatal (White *et al*., 1990), attenuates such effects.

In the present study, we have investigated the effects of systemic, intrastriatal or intranigral d-amphetamine, on AA, glutamate, aspartate and uric acid release in the striatum of freely-moving rats, in order to clarify the mechanism by which d-amphetamine affects these striatal neurochemical parameters. In addition, we describe the effects of systemic, intrastriatal or intranigral d-amphetamine on DA release and oxidative metabolism.

Methods

Animals

Male Wistar rats (Morini R. Emilia, Italy), weighing 280–330 g were used in all experiments. The rats were maintained under standard animal care conditions (12 : 12 h light/dark cycle, light at 07.00 h; room temperature 21°C), with food and water *ad libitum*. Prior to the start of any experiment, the health of the rat was assessed according to published guidelines (Morton & Griffiths, 1985). All procedures were specifically licensed under the European Community directive 86/609 included in Decreto No. 116/1992 of the Italian Ministry of Public Health.

Drugs

The following drugs were used: d-amphetamine sulphate (Merck AG, Darmstadt, Germany); haloperidol (Sigma-Aldrich, Milano, Italy). Subcutaneous (s.c.) d-amphetamine 2 mg kg^{-1} was given in 2 ml kg^{-1} of saline (0.9%). The dose was that used in the previous study (Desole *et al*., 1992). The study of the saline (2 ml kg^{-1} s.c.) effect on striatal neurochemical concentrations was omitted, since the lack of s.c. saline effect on striatal dialysate concentrations of neurochemicals has been discussed in a previous study (Enrico *et al*., 1997). For intrastriatal or intranigral administration, d-amphetamine was dissolved to 0.2 mM in the artificial cerebrospinal fluid (aCSF) and infused for 15 min. For intrastriatal administration, haloperidol (0.1 mM) in aCSF was infused for 15 min. The haloperidol concentration was in the range of those used by Biggs *et al*. (1997).

Microdialysis probe construction

The striatal probe combined two independent microdialysis probes of concentric design with two separate inlets and a shared outlet (Figure 1). The probes were constructed using two sections of plastic-coated silica tubing (ø 0.15 mm; Scientific Glass Engineering, Milton Keynes, U.K.) each placed in the centre of a semi-permeable polyacrylonitrile dialysis fibre (molecular cut-off weight of 12 KD, Filtral 16 Hospal Industrie, France). Each probe had a final diameter of 0.22 mm. The tips of the dialysis fibres were sealed and joined using quick-drying epoxy glue. The two silica tubes served as inlets; the outlet was made also with a section of plastic-coated silica tubing, positioned in the centre of polythene tubing. The semi-permeable membrane was coated with epoxy glue leaving an active length of 4 mm. The diameter of the final probe was approximately 0.5 mm. The probe allowed us to collect a volume of dialysate enough to permit simultaneous measurement of DA, metabolites and amino acids without increasing the flow rate of perfusion or the interval sampling time. In a separate set of experiments, a probe was implanted on its own in the right *substantia nigra*, to allow local infusion of drugs, whereas dialysate samples were collected by means of the striatal probe.

Surgery

Stereotaxic surgery was performed under chloral hydrate (400 mg kg^{-1} i.p.) anaesthesia. The microdialysis probe was implanted in the right striatum using the following coordinates from the atlas of Paxinos & Watson (1986): A/P +0.5 from bregma, +2.5 M/L, and −6.0 D/V from dura. In a separate group of animals the probe was implanted in the right SN using the following coordinates: A/P −5.3 from bregma, +2.2 M/L, and −7.7 D/V from dura. Body temperature during anaesthesia was maintained at 37°C by means of an isothermal heating pad. Following surgery, the animals were placed in large plastic bowls (50 × 55 cm), and maintained in a temperature- and light-controlled environment, with free access to food and water. Experiments were carried out 24 h after probe implantation with the animal in its home bowl. This arrangement allowed the rats free movement.

Microdialysis procedure

The composition of the aCSF used was as follows, in mM: NaCl 147.0, KCl 4.0, $CaCl_2$ 1.2, $MgCl_2$ 1.0. A microinfusion pump (CMA/100, Microdialysis, Sweden) pumped aCSF

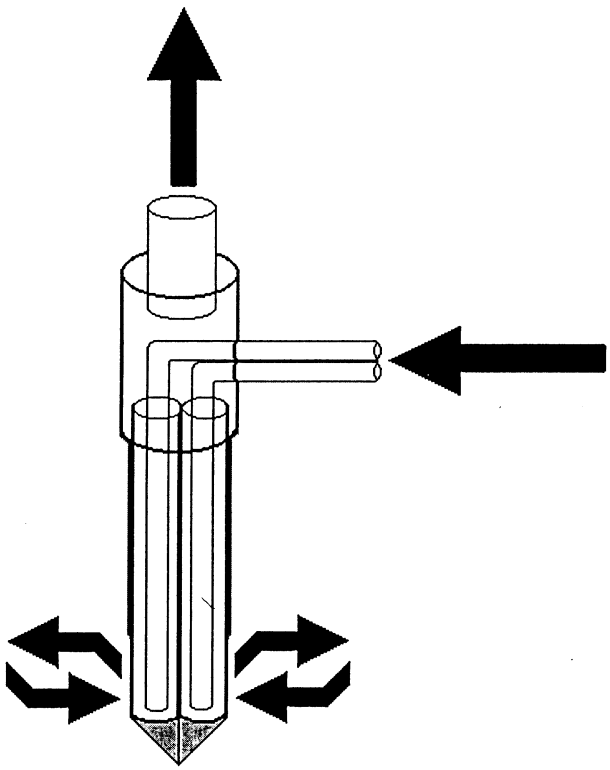

Figure 1 Drawing of a striatal probe combining two independent microdialysis probes of concentric design with two separate inlets and a shared outlet. Each probe has a final diameter of 0.22 mm. The semi-permeable membrane has an active length of 4 mm. The diameter of the final probe is approximately 0.5 mm.

into the probe at a flow rate of 1.5 μl min^{-1} using two separate syringes connected to the inlets by a length of polythene tubing. Every 20 min, 60 μl dialysate samples were collected manually in 250 μl micro-centrifuge tubes (Alpha Laboratories, U.K.) attached to the outlet. Subsequently, two 20 μl aliquots of collected dialysate were injected separately into each of two parallel analytical systems. The first sample was collected after a 40 min period of stabilization (time 0), then dialysates were collected, at 20 min intervals, for 1 h prior to the start of experiments.

Chromatographic analysis

AA, uric acid, DA, DOPAC, HVA and 5-HIAA were quantified by HPLC electrochemical detection using an Alltech 426 pump equipped with a Rheodyne injector, column 15 × 4.6 mm i.d. Alltech Adsorbsphere C18 5 μm, electrochemical detector Antec CU-04-AZ and Varian Star Chromatographic Workstation. The mobile phase was citric acid 0.5 M, Na acetate 1 M, EDTA 12.5 mM, MeOH 10% and sodium octylsulphate 650 mg l^{-1} (pH = 3.0); the flow rate was 1.3 ml min^{-1}. Dialysates were injected directly into the HPLC.

Glutamate and aspartate were quantified by HPLC with fluorimetric detection (at 360 and 450 nm – excitation and emission wavelengths) using a Varian 9010 gradient system following precolumn derivatization by o-phthaldialdeyde. Derivatized glutamate and aspartate were separated on Ultrasphere C 18 3 μm high speed 75 × 4.6 mm Beckman column. A gradient was run from 25 mM Na_2HPO_4-acetonitril (95 : 5) pH 6.6 with H_3PO_4 to 25 mM Na_2HPO_4-acetonitril (70 : 30) in 20 min at a flow rate of 1.2 ml min^{-1}.

Histology

Following the experiments, rats were killed with an overdose of chloral hydrate (800 mg kg^{-1} i.p.). The location of each microdialysis probe was confirmed by post-mortem histology. Brains were fixed in formal saline and 50 μm coronal sections were made with a cryostat. The slices were stained with cresyl violet and examined under a microscope.

Statistical analysis

The concentrations in the dialysate were expressed in nM (DA) or μM (DOPAC + HVA, 5-HIAA, glutamate, aspartate, AA and uric acid) and given as mean ± s.e.mean. Drug effects on neurochemicals were evaluated as changes from the preceding baseline. For this purpose data are expressed as percentage of baseline. Statistical significance was assessed using analysis of variance (ANOVA) per difference over time. Differences within groups were determined by paired *t*-tests, with the Bonferroni multiple comparison adjustment. Pearson's correlation coefficient between individual DA and DOPAC + HVA, DA and glutamate, or glutamate and AA dialysate concentrations, was calculated in each rat after each d-amphetamine administration.

Results

Baseline dialysate concentrations of neurochemicals

Baseline dialysate concentrations of neurochemicals detected in each experimental group are given in Table 1.

Effects of subcutaneous d-amphetamine

d-Amphetamine (2 mg kg^{-1}, $n = 5$), increased (ANOVA $P < 0.001$) dialysate levels of DA (maximum increase to 912% of baseline after 40 min), AA (maximum increase to 150% of baseline after 160 min) and uric acid (maximum increase to 155% of baseline after 180 min). Conversely, d-amphetamine decreased (ANOVA $P < 0.001$) dialysate levels of DOPAC + HVA (maximum decrease to 46% of baseline after 100 min), glutamate (maximum decrease to 47% of baseline after 120 min), aspartate (maximum decrease to 52% of baseline after 100 min), while 5-HIAA levels were unaffected (Figure 2).

Effects of intrastriatal d-amphetamine or haloperidol

d-Amphetamine (0.2 mM, $n = 5$) increased (ANOVA $P < 0.001$) dialysate levels of DA (maximum increase to 1511% of baseline after 20 min) and decreased those of DOPAC + HVA (maximum decrease to 77% of baseline after 40 min) and aspartate (maximum decrease to 64% of baseline after 20 min), while 5-HIAA, uric acid, glutamate and AA and levels were unaffected (Figure 3). At a higher concentration (2 mM), d-amphetamine greatly increased peak (up to > 2000% of baseline) and duration (120 min) of DA overflow, decreased DOPAC + HVA and aspartate concentration, but did not modify AA, glutamate or uric acid concentrations (data not shown).

The unexpected finding that intrastriatal d-amphetamine resulted in a decrease in aspartate dialysate levels prompted us to study the effect of intrastriatal haloperidol, a preferential D_2-like receptor antagonist. In rats given haloperidol (0.1 mM, $n = 4$), baseline dialysate AA, glutamate and aspartate concentrations were 5.37 ± 1.60, 3.35 ± 0.86 and 0.23 ± 0.26 μM, respectively. Haloperidol increased (ANOVA $P < 0.001$) dialysate levels of aspartate (maximum increase to 217% of baseline after 80 min), but did not modify AA or glutamate dialysate concentrations (Figure 4).

Effects of intranigral d-amphetamine

d-Amphetamine (0.2 mM, $n = 5$), induced a short-lasting decrease (ANOVA $P < 0.02$) in dialysate DA levels (maximum decrease to 78% of baseline after 40 min). Also, dialysate glutamate and aspartate levels were decreased (ANOVA $P < 0.001$) to a maximum of 80% (after 40 min) and 65% (after 80 min) of baseline, respectively, while AA and uric acid levels were increased (ANOVA $P < 0.01$) to a maximum of 140% of baseline (after 20 min) and 146% of baseline (after

Table 1 Baseline striatal levels of neurochemicals detected in each experimental group given d-amphetamine

	Systematic ($n = 5$)	*Intrastriatal* ($n = 5$)	*Intranigral* ($n = 5$)	*ANOVA* P
Glutamate (μM)	4.43 ± 1.0	5.64 ± 1.61	3.88 ± 1.03	> 0.2
Aspartate (μM)	0.46 ± 0.09	0.51 ± 0.09	0.37 ± 0.07	> 0.2
AA (μM)	5.39 ± 0.72	5.89 ± 0.64	4.30 ± 0.58	> 0.2
Uric acid (μM)	1.13 ± 0.18	1.87 ± 0.26	1.82 ± 0.15	> 0.1
DA (nM)	3.55 ± 0.46	3.46 ± 0.59	5.37 ± 2.14	> 0.1
DOPAC + HVA (μM)	1.47 ± 0.25	1.12 ± 0.17	1.79 ± 0.25	> 0.5
5-HIAA (μM)	0.36 ± 0.02	0.27 ± 0.06	0.42 ± 0.06	> 0.1

Data are given as mean ± s.e.mean.

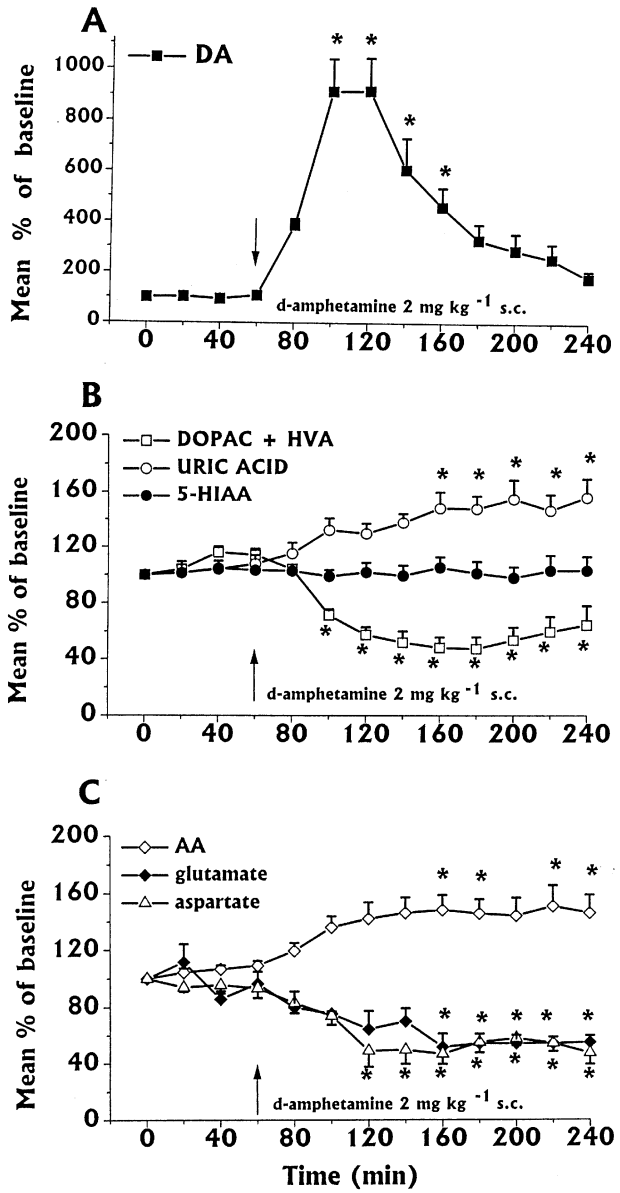

Figure 2 Effect of s. c. administration of d-amphetamine (arrow) on DA (A), DOPAC + HVA, 5-HIAA and uric acid (B), glutamate, aspartate and AA (C) dialysate concentrations. Dialysates were collected, at 20 min intervals, for 180 min after d-amphetamine administration. Data are expressed as percentage of baseline values and are given as mean ± s.e.mean ($n = 5$). Baseline dialysate concentrations were: DA 3.55 ± 0.46 nM, DOPAC + HVA 1.47 ± 0.25 μM, 5-HIAA 0.36 ± 0.02 μM, uric acid 1.13 ± 0.18 μM, glutamate 4.43 ± 1.0 μM aspartate 0.46 ± 0.09 μM, AA 5.39 ± 0.72 μM. Statistical significance (ANOVA followed by the Bonferroni multiple comparison adjustment) $^*P < 0.05$ compared with the baseline values.

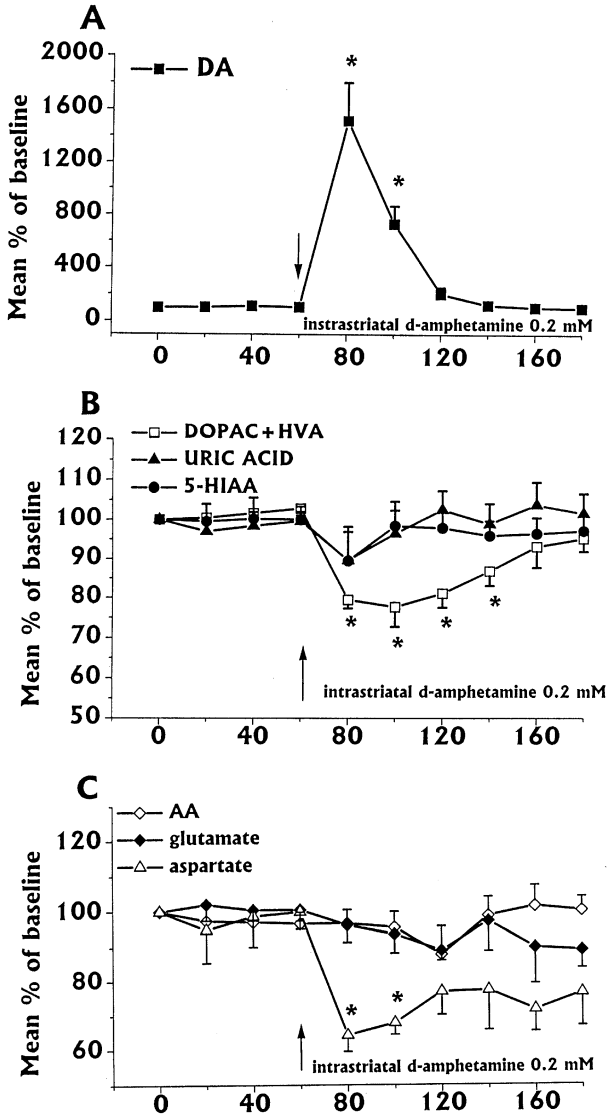

Figure 3 Effect of intrastriatal administration of d-amphetamine (arrow) on DA (A), DOPAC + HVA, 5-HIAA and uric acid (B), glutamate, aspartate and AA (C) dialysate concentrations. Dialysates samples were collected, at 20 min intervals, for 120 min after d-amphetamine administration. Data are expressed as percentage of the baseline values and are given as mean ± s.e.mean ($n = 5$). Baseline dialysate concentrations were: DA 3.46 ± 0.59 nM, DOPAC + HVA 1.12 ± 0.17 μM, 5-HIAA 0.27 ± 0.06 μM, uric acid 1.87 ± 0.26 μM, glutamate 5.64 ± 1.61 μM, aspartate 0.51 ± 0.09 μM and AA 5.89 ± 0.64 μM. Statistical significance (ANOVA followed by the Bonferroni multiple comparison adjustment) $^*P < 0.05$ compared with baseline values.

120 min), respectively. DOPAC + HVA and 5-HIAA levels were unaffected (Figure 5).

Correlations between individual DA and DOPAC + HVA, DA and glutamate or glutamate and AA concentrations

Pearson's correlation coefficient was calculated between individual DA and DOPAC + HVA, DA and glutamate or glutamate and AA dialysate concentrations detected in each rat before and after each d-amphetamine administration.

In all rats given d-amphetamine s.c (2 mg kg^{-1}, $n = 6$), no significant correlation was found between individual DA and DOPAC + HVA concentrations (r value range between -0.251, $P > 0.4$ and -0.441, $P > 0.1$, df = 11) or between DA and glutamate concentrations (r value range between -0.224, $P > 0.4$ and -0.468, $P > 0.1$, df = 11). Conversely, individual glutamate concentrations were negatively correlated with AA concentrations in all rats (r value range between -0.637, $P < 0.05$ and -0.885, $P < 0.001$, df = 11)

In all rats given d-amphetamine (0.2 mM) intrastriatally ($n = 5$), no significant correlation was found between individual DA and DOPAC + HVA concentrations (r value range between -0.295, $P > 0.5$, and -0.552, $P > 0.1$, df = 8) or between DA and glutamate concentrations (r value range

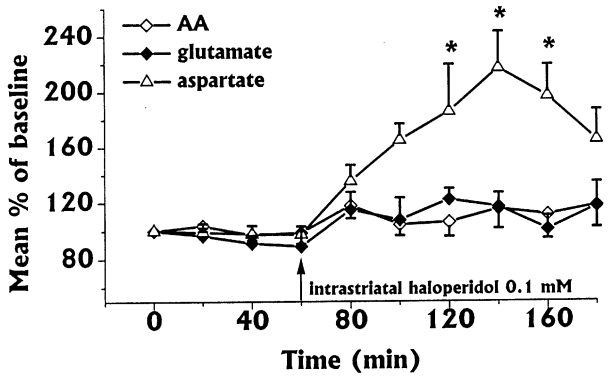

Figure 4 Effect of intrastriatal administration of haloperidol (arrow) on AA, glutamate and aspartate dialysate concentrations. Dialysates were collected, at 20 min intervals, for 120 min after haloperidol administration. Data are expressed as percentage of the baseline values and are given as mean ± s.e.mean ($n=4$). Baseline dialysate concentrations were: AA 5.37 ± 1.60 μM, glutamate 3.35 ± 0.86 μM and aspartate 0.23 ± 0.26 μM concentrations. Statistical significance (ANOVA followed by the Bonferroni multiple comparison adjustment) $^*P<0.05$ compared with baseline values.

between −0.611, $P>0.08$ and +0.577, $P>0.1$, df = 8). Negative correlation between individual glutamate and AA concentrations was found only in two out of five rats (r value −0.675 and −0.713, respectively, $P<0.05$).

In all rats given haloperidol (0.1 mM) intrastriatally ($n=5$), no significant correlation was found between individual AA and glutamate concentrations.

In all rats given d-amphetamine intranigrally (0.2 mM, $n=5$), no significant correlation was found between individual DA and DOPAC + HVA concentrations or between DA and glutamate concentrations. Conversely, individual glutamate concentrations were negatively correlated with AA concentrations (r value range between −0.656, $P<0.05$ and −0.809, $P<0.01$, df = 8).

Behavioural changes

Systemic (s.c.) or intrastriatal d-amphetamine both induced increases in motor activity and stereotypy (*c.f.* Desole *et al.*, 1992). Conversely, intranigral d-amphetamine did not induce behavioural changes.

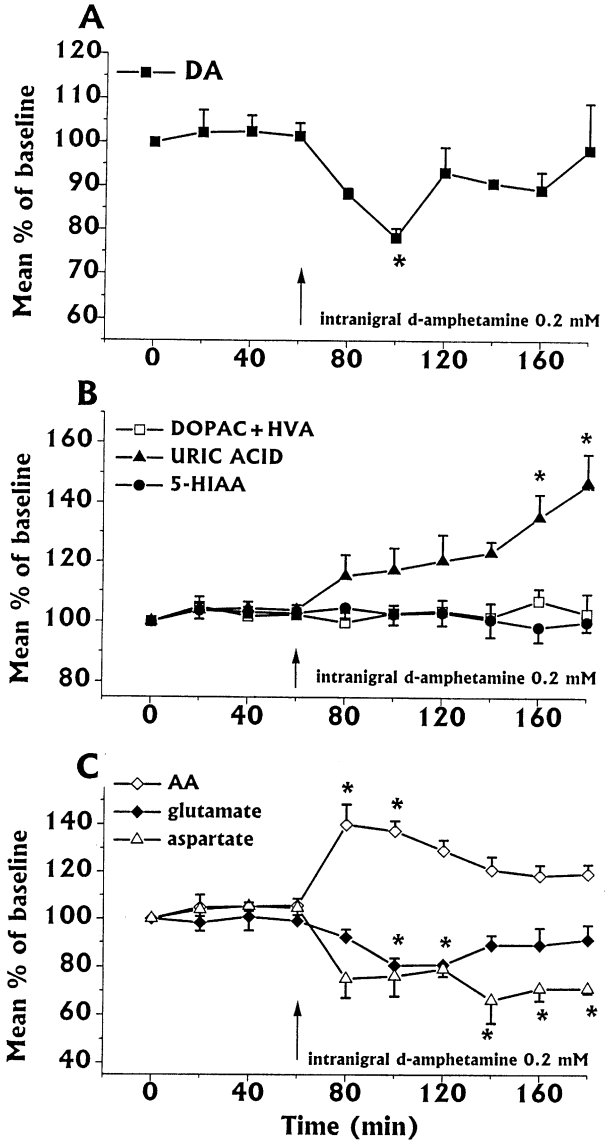

Figure 5 Effect of intranigral administration of d-amphetamine (arrow) on DA (A), DOPAC + HVA, 5-HIAA and uric acid (B), glutamate, aspartate and AA (C) dialysate concentrations. Dialysates were collected, at 20 min intervals, for 120 min after d-amphetamine administration. Data are expressed as percentage of the baseline values and are given as mean ± s.e.mean ($n=5$). Baseline dialysate concentrations were: DA 5.37 ± 2.14 nM, DOPAC + HVA 1.79 ± 0.25 μM, 5-HIAA, 0.42 ± 0.06 μM, uric acid 1.82 ± 0.15 μM, glutamate 3.88 ± 1.03 μM, aspartate 0.37 ± 0.07 μM, AA 4.30 ± 0.58 μM. Statistical significance (ANOVA followed by the Bonferroni multiple comparison adjustment) $^*P<0.05$ compared with baseline values.

Discussion

It is well known that d-amphetamine increases DA release in terminal fields of dopaminergic neurones. Both vesicular and newly-synthesized DA appear to be released by d-amphetamine into the striatal extracellular compartment (Cadoni *et al.*, 1995; Jones *et al.*, 1998). d-Amphetamine also decreases DA oxidative metabolism by inhibiting MAO (Jones *et al.*, 1998) and depletion of newly-synthesized DA, the main substrate for MAO, has been suggested as an additional mechanism (Zetterström *et al.*, 1986). In the present study, systemic d-amphetamine, as expected, increased DA and decreased its acidic metabolite levels in the striatal dialysate from freely-moving rats. The increase in DA release (8–9 fold of baseline levels) was in agreement with the literature data (Robertson *et al.*, 1991). Increases in DA and decreases in acidic metabolite dialysate concentrations were observed also when d-amphetamine was given intrastriatally: the peak of DA increase was greater and occurred earlier than systemic d-amphetamine, while the peak of DOPAC + HVA decrease also occurred earlier but was lower than that of systemic d-amphetamine. Intranigral infusion of d-amphetamine decreases DA release in the ipsilateral striatum (Wilson & Wingthman, 1985; Pierce *et al.*, 1994). This effect is the result of nigral dendritic DA release (Robertson *et al.*, 1991) and consequent activation of inhibitory somatodendritic autoreceptors (Westerink *et al.*, 1994). In the present study, intranigral d-amphetamine induced a short-lasting decrease in DA release but did not affect DA oxidative metabolism. Zetterström *et al.* (1986) found a statistically significant correlation between the increase in DA release and the decrease in DOPAC level after systemic d-amphetamine, suggesting that the two effects might share a common mechanism. In the present study, no significant correlation between the increase in extracellular

DA and the decrease in DOPAC + HVA concentrations was found following systemic, intrastriatal or intranigral d-amphetamine. These findings clearly indicate that its effects on DA release and DA oxidative metabolism are dissociated events. No tested route of d-amphetamine affected striatal 5-HT oxidative metabolism, as judged by 5-HIAA concentrations. These findings are in agreement with the literature (Wan *et al*., 1996).

In a previous study, we showed that morphine-induced increases in DA oxidative metabolism are highly correlated with the increase in xanthine oxidative metabolism (Enrico *et al*., 1997). Superoxide radicals generated from xanthine oxidation may participate in the enzymatic DA oxidation according to the following scheme (Chiueh, 1994): O_2 + xanthine (XO) → uric acid + O_2^- + DA (MAO) → DOPAC + H_2O_2 It is likely that d-amphetamine does not increase xanthine oxidative metabolism, but simply mobilizes uric acid from intraneuronal pools (Mueller & Kunko, 1990) acting at nigral sites. This hypothesis is supported by the moderate d-amphetamine-induced increase in uric acid levels, which is consistent with a mobilization from an intraneuronal pool.

The reciprocal dopamine-glutamate modulation of release in the basal ganglia (see Morari *et al*., 1998) suggests a direct interaction between striatal dopaminergic and glutamatergic terminals. Inhibitory dopaminergic receptors on striatal glutamatergic terminals reportedly regulate glutamate release (Yamamoto & Davy, 1992). In contrast, Reid *et al*. (1997) have shown that dopaminergic stimulants do not affect glutamate release in the striatum, but enhance it in limbic brain structures including the nucleus accumbens and prefrontal cortex. In the present study, the increase in dialysate DA concentrations up to 15 times of baseline induced by d-amphetamine given intrastriatally did not induce a change in dialysate glutamate concentrations. Thus, the decrease in glutamate dialysate concentration induced by systemic d-amphetamine appears to be unrelated to the great increase in DA extracellular levels induced by systemic d-amphetamine. Conversely, d-amphetamine given intrastriatally significantly decreased aspartate dialysate levels. The decrease is likely to be mediated by D_2-receptors, since the D_2-selective antagonist haloperidol, when given intrastriatally, increased aspartate levels without affecting those of AA and glutamate. This hypothesis is supported by the findings of Biggs *et al*. (1997), which showed that haloperidol increases aspartate release in the entopeduncular nucleus of the rat. In addition, Labarca *et al*. (1995) showed that d-amphetamine inhibits K^+-induced release of aspartate in nucleus accumbens of the rat, an effect which is antagonized by haloperidol. K^+-induced release of aspartate might be also modulated by D_1-receptors, since Yamamoto & Davy (1992) showed that D_1-agonists inhibit K^+-induced release of aspartate, but not of glutamate, in the striatum of freely moving rats.

Extracellular glutamate levels monitored by *in vivo* microdialysis reflect the balance between neuronal/glial release and reuptake into surrounding nerve terminals and glial elements (Herrera-Marschitz *et al*., 1996). In the present study, systemic or intranigral, but not intrastriatal d-amphetamine, induced a decrease in dialysate glutamate levels.

Wilson & Wingthman (1985) first reported that direct infusion of d-amphetamine in the substantia nigra of anaesthetized rats induced striatal ascorbate release. The finding has also been confirmed also in freely moving animals (Pierce *et al*., 1994). It has been proposed that a neuronal circuit, which includes activation of SN reticulata, disinhibition of thalamocortical neurons and, ultimately, activation of glutamatergic corticostriatal fibres, controls d-amphetamine induced striatal AA release (Basse-Tomusk & Rebec, 1990; Pierce *et al*., 1994; Rebec & Pierce, 1994). In the present study, when given intranigrally, d-amphetamine increased AA and decreased glutamate dialysate concentrations. Individual dialysate AA concentrations were negatively correlated with those of glutamate. Such correlation is consistent with the functioning of a glutamate/ascorbate heteroexchange system (O'Neill, 1995), in which ascorbate release is linked to impulse traffic, transmitter release and glutamate uptake (Miele *et al*., 1994). The AA source may be glial as well as neuronal (O'Neill *et al*., 1984; Cammack *et al*., 1991). The mechanism by which d-amphetamine, acting at nigral sites, activates a neuronal circuit which ultimately induces an increase in striatal glutamate uptake and consequent AA release remains to be elucidated. Mennerick *et al*. (1996) have shown, in microculture of hippocampal neurons, that all the released neuronal glutamate is rapidly cleared by transport into glial cells. Thus, it is likely that d-amphetamine-induced activation of the intrastriatal glutamatergic system includes both neuronal glutamate release and glial/neuronal uptake.

In conclusion: (i) d-amphetamine increases DA release and inhibits DA oxidative metabolism acting at striatal sites; (ii) the changes in glutamate, AA and uric acid extracellular striatal concentrations are triggered at nigral sites; (iii) changes in striatal aspartate can result from at least two mechanisms: striatal (mediated by inhibitory dopaminergic receptors) and nigral (activation of amino acid carrier-mediated uptake). In addition, the negative correlation between d-amphetamine-induced changes in AA and glutamate support the glutamate-AA heteroexchange model for striatal AA release.

The research was supported by University of Sassari (ex 60% quota, 1999) and CNR contributo 97.04482.CT04.

References

BASSE-TOMUSK, A. & REBEC, C.V. (1990). Corticostriatal and thalamic regulation of amphetamine-induced ascorbate release in neostriatum. *Pharmacol. Biochem. Behav.,* **35,** 55 – 60.

BIGGS, C.S., FOWLER, L.J., WHITTON, P.S. & STARR, M.S. (1997). Extracellular levels of glutamate and aspartate in the entopeduncular nucleus of the rat determined by micro-dialysis: regulation by striatal dopamine D_2 receptors via the indirect striatal output pathway? *Brain Res.,* **753,** 163 – 175.

CADONI, C., PINNA, A., RUSSI, G., CONSOLO, G. & DI CHIARA, G. (1995). Role of vesicular dopamine in the in vivo stimulation of striatal dopamine transmission by amphetamine: evidence from microdialysis and Fos immunochemistry. *Neuroscience,* **65,** 1027 – 1039.

CAMMACK, J., GHASEMZADEH, B. & ADAMS, R.N. (1991). The pharmacological profile of glutamate-evoked ascorbate efflux measured by in vivo electrochemistry. *Brain Res.,* **565,** 17 – 22.

CHIUEH, C.C. (1994). Auto-oxidation of dopamine and formation of free radicals *in vivo*. In: Louilot, A., Durkin, A.T., Spampinato, U. & Cador, M. (eds.). *Monitoring Molecules in Neuroscience*. Universities of Bordeaux I & II. pp. 127 – 128.

CHURCH, W.H. & WARD, V.L. (1994). Uric acid is reduced in the substantia nigra in Parkinson's disease: effect on dopamine autoxidation. *Brain Res. Bull.,* **33,** 419 – 425.

DESOLE, M.S., ANANIA, V., ESPOSITO, G., CARBONI, F., SENINI, A. & MIELE, E. (1987). Neurochemical and behavioural changes induced by ascorbic acid and d-amphetamine in the rat. *Pharmacol. Res. Comm.,* **19,** 441–450.

DESOLE, M.S., MIELE, M., ENRICO, P., FRESU, L., ESPOSITO, G., DE NATALE, G. & MIELE, E. (1992). The effects of cortical ablation on d-amphetamine-induced changes in striatal dopamine turnover and ascorbic acid catabolism in the rat. *Neurosci. Lett.,* **139,** 29–33.

ENRICO, P., ESPOSITO, G., MURA, M.A., MIGHELI, R., SERRA, P.A., DESOLE, M.S., MIELE, E., DE NATALE, G. & MIELE, M. (1997). Effects of allopurinol on striatal dopamine, ascorbate and uric acid during an acute morphine challenge: ex vivo and in vivo studies. *Pharmacol. Res.,* **35,** 577–585.

HERRERA-MARSCHITZ, M., YOU, Z.B., GOINY, M., MEANA, J.J., SILVEIR, R., GODUKIN, O.V., ESPINOZA, S., PETTERSON, E., LOIDL, C.F., LUBEC, G., ANDERSONN, K., NYLANDER, I., TERENIUS, L. & UNGERSTEDT, U. (1996). On the origin of extracellular glutamate levels monitored in the basal ganglia of the rat by in vivo microdialysis. *J. Neurochem.,* **66,** 1726–1735.

JONES, S.R., GAINETDINOV, R.R., WINGTHMAN, R.M. & CARON, M.G. (1998). Mechanism of amphetamine action as revealed in mice lacking the dopamine transporter. *J. Neurosci.,* **18,** 1979–1986.

KARLER, K., CALDER, L.D., THAI, L.H. & BEDINGFIELD, J.B. (1995). The dopaminergic, glutamatergic, GABAergic bases for the action of amphetamine and cocaine. *Brain. Res.,* **671,** 100–104.

LABARCA, R., GAJARDO, M.I., SEGUEL, M., SILVA, H., JEREZ, S., RUIZ, A. & BUSTOS, G. (1995). Effects of d-amphetamine administration on the release of endogenous excitatory amino acids in the rat nucleus accumbens. *Progr, Neuropsychoph.,* **19,** 467–473.

MAJEWSKA, M.D., BELL, J.A. & LONDON, E.D. (1990). Regulation of NMDA receptor by redox phenomena:inhibitory role of ascorbate. *Brain Res.,* **537,** 328–332.

MENNERICK, S., BENZ, A. & ZORUMSLY, C.F. (1996). Components of glial response to exogenous and synaptic glutamate in rat hippocampal microcultures. *J. Neurosci.,* **16,** 55–64.

MIELE, M., BOUTELLE, M.G. & FILLENZ, M. (1994). The physiologically-induced release of ascorbate in rat brain is dependent on impulse traffic, calcium influx and glutamate uptake. *Neuroscience,* **62,** 87–91.

MIELE, M. & FILLENZ, M. (1996). In vivo determination of extracellular brain ascorbate. *J. Neurosci. Meth.,* **70,** 15–19.

MORARI, M., MARTI, M., SBRENNA, S., FUXE, K., BIANCHI, C. & BEANI, L. (1998). Reciprocal dopamine-glutamate modulation of release in basal ganglia. *Neurochem. Int.,* **33,** 383–397.

MORTON, D.B. & GRIFFITHS, P.H.M. (1985). Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. *Vet. Records,* **116,** 431–436.

MUELLER, K. & KUNKO, P.M. (1990). The effects of amphetamine and pilocarpine on the release of ascorbic acid and uric acid in several rat brain areas. *Pharmacol. Biochem. Behav.,* **35,** 871–876.

OHMORI, T., ABEKAWA, T. & KOYAMA, T. (1996). The role of glutamate in the behavioral effects of methamphetamine. *Neurochem. Int.,* **29,** 301–307.

O'NEILL, R.D. (1995). The measurement of brain ascorbate in vivo and its link with excitatory amino acid neurotransmission. In: Boulton, A., Baker, G. & Adams, R.N. (eds.). *Neurometods, Vol. 27 Voltammetric Methods in Brain Systems*. Humana Press Inc. pp. 221–268.

O'NEILL, R.D., FILLENZ, M., SUNDSTROM, L. & RAWLINS, J.N.P. (1984). Voltammetrically monitored brain ascorbate as an index of excitatory amino acid release in the unrstrained rat. *Neurosci. Lett.,* **52,** 227–233.

PAXINOS, G. & WATSON, C. (1986). *Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego.

PIERCE, R.C., CLEMENT, J.A., GRABNER, C.P. & REBEC, G.V. (1994). Amphetamine promotes neostriatal ascorbate release via a nigrotalamo-cortico-neostriatal loop. *J. Neurochem.,* **63,** 1499–1507.

REBEC, G.V., CENTORE, J.M., WHITE, L.K. & ALLOWAY, K.D. (1985). Ascorbic acid and the behavioral response to haloperidol: implications for the action of antipsychotic drugs. *Science,* **227,** 438–440.

REBEC, G.V. & PIERCE, C.P. (1994). A vitamin as neuro-modulator: ascorbate release into the extracellular fluid of the brain regulates dopaminergic and glutamatergic transmission. *Progr. Neurobiol.,* **43,** 537–565.

REID, M.S., HSU JR K. & BERGER, S.P. (1997). Cocaine and amphetamine preferentially stimulate glutamate release in limbic system: studies on the involvement of dopamin. *Synapse,* **27,** 95–105.

ROBERTSON, G.S., DAMSMA, G. & FIBIGER, H.C. (1991). Characterization of dopamine release in the substantia nigra by in vivo microdialysis in freely moving rats. *J. Neurosci.,* **11,** 2209–2216.

ROBINSON, T.E. & BECKER, J.B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. *Brain Res. Rev.,* **11,** 1067–1079.

SAPONJIC, R.M., MUELLER, K., KRUG, D. & KUNKO, P.M. (1994). The effects of haloperidol, scopolamine, and MK-801 on amphetamine-induced increase in ascorbic acid and uric acid as determined by voltammetry in vivo. *Pharmacol. Biochem. Behav.,* **48,** 161–168.

SEVANIAN, A., DAVIES, K.J.A. & HOCHSTEIN, P. (1991). Serum urate as an antioxidant for ascorbic acid. *Am. J. Clin. Nutr.,* **54,** 129S–134S.

SULZER, D., CHEN, T-K., LAU, Y.Y., KRISTENSEN, H., RAYPORT, S. & EWING, A. (1995). Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J. Neurosci.,* **15,** 4102–4108.

WAN, R.Q., GIOVANNI, A., KAFKA, S.H. & CORBETT, R. (1996). Neonatal ippocampal lesions-induced hyperresponsiveness to amphetamine: behavioral and in vivo microdialysis studies. *Behav. Brain. Res.,* **78,** 211–223.

WESTERINK, B.H.C., DE BOER, P., SANTIAGO, M. & DE VRIES, J.B. (1994). Do nerve terminals and cell bodies of nigrostriatal dopaminergic neurons of the rat contain similar receptors? *Neurosci. Lett.,* **167,** 109–112.

WHITE, L.K., MAURER, M.A., SIDELL, E.A., KRAFT, M.E., OH, C. & REBEC, G.V. (1990). Intrastriatal infusion of ascorbate antagonizes the behavioral response to amphetamine. *Pharmacol. Biochem. Behav.,* **36,** 485–489.

WILSON, R.L. & WINGTHMAN, R.M. (1985). Systemic and nigral application of amphetamine both cause an increase of extracellular concentrations of ascorbate in the caudate nucleus of the rat. *Brain Res.,* **370,** 219–226.

YAMAMOTO, B.K. & DAVY, M. (1992). Dopaminergic modulation of glutamate release in striatum as measured by microdialysis. *J. Neurochem.,* **58,** 1736–1742.

ZETTERSTRÖM, T. & FILLENZ, M. (1988). Changes in striatal dopamine, ascorbic acid and motor activity in rats after administration of dopamine receptor agonists. *Brit. J. Pharmacol.,* **93,** p. 149.

ZETTERSTRÖM, T., SHARO, T. & UNGERSTEDT, U. (1986). Further evaluation of the mechanism by which amphetamine reduces striatal dopamine metabolism: a brain dialysis study. *Eur. J. Pharmacol.,* **132,** 1–9.

(Received August 8, 1999
Revised October 15, 1999
Accepted October 27, 1999)