ELSEVIER

European Journal of Pharmacology 424 (2001) 115–119

ejp

www.elsevier.nl/locate/ejphar

Short communication

Tyrosine depletion attenuates the behavioural stimulant effects of amphetamine and cocaine in rats

Sarah F.B. McTavish [a], Ben Raumann [b], Philip J. Cowen [a], Trevor Sharp [b,*]

[a] *University Department of Psychiatry, Warneford Hospital, Oxford, UK*
[b] *Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK*

Received 22 February 2001; received in revised form 29 May 2001; accepted 1 June 2001

Abstract

Neurochemical studies show that a tyrosine-free amino acid mixture depletes brain tyrosine and decreases dopamine synthesis and release. Here, we tested whether such a mixture would reduce the behavioural effects of amphetamine and other psychostimulants. A tyrosine-free amino acid mixture decreased the behavioural activation induced by both D-amphetamine (2 mg/kg s.c.) and cocaine (2 mg/kg s.c.). In contrast, the activation induced by the dopamine agonist, apomorphine (0.75 and 5 mg/kg s.c.), or the 5-hydroxytryptamine releasing agent, *p*-chloroamphetamine (2 mg/kg s.c.) was not altered. These findings provide behavioural evidence that tyrosine-free amino acid mixtures reduce presynaptic dopamine function in the brain.

Keywords: Tyrosine depletion; Dopamine; Amphetamine; Cocaine

1. Introduction

The synthesis of brain catecholamines is dependent on the supply of the essential amino acid, L-tyrosine via a large neutral amino acid transporter. Strategies aimed to reduce tyrosine availability are currently being developed as a novel means to manipulate catecholamine function for psychopharmacological investigations, and as a potential therapy in psychiatric states in which an elevation of brain catecholamines is implicated.

One strategy to deplete tyrosine involves administration of an amino acid mixture lacking tyrosine. This manipulation results in a decrease in plasma and brain tyrosine levels (Biggio et al., 1976; Moja et al., 1996) and a reduction in the synthesis of catecholamines in the brain (Fernstrom and Fernstrom, 1995; McTavish et al., 1999b). Recent neurochemical studies indicate that brain dopamine systems are particularly vulnerable to tyrosine depletion. Thus, a tyrosine-free amino acid mixture reduces in ex vivo catecholamine synthesis in regions with a predominantly dopaminergic innervation (striatum and nucleus accumbens) and attenuates amphetamine-induced release of dopamine in microdialysis experiments on the anesthetized rat (McTavish et al., 1999b). In comparison, catecholamine synthesis in regions with a predominantly noradrenergic innervation (cortex, hippocampus and hypothalamus) was less affected. Moreover, in microdialysis experiments, the tyrosine free amino acid load did not attenuate the release of noradrenaline evoked by either amphetamine or the α_2-adrenoceptor antagonist, idazoxan (McTavish et al., 1999a,b).

Pharmacological manipulations that decrease dopamine synthesis and release would be predicted to inhibit the behavioural activation induced by dopamine releasing agents and dopamine reuptake inhibitors (e.g. Widerlov and Levander, 1978; Le et al., 1997). Here, we tested whether a tyrosine-free amino acid mixture attenuates the behavioural activation induced by amphetamine and cocaine. To rule out non-specific behavioural effects, the action of the amino acid mixture on behaviour induced by the direct acting dopamine agonist, apomorphine, and the 5-hydroxytryptamine (5-HT) releasing agent, *p*-chloroamphetamine, was also examined.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 225–300 g (Harlan-Olac, Bicester, UK) were used throughout. Rats

* Corresponding author. Tel.: +44-1865-271-850; fax: +44-1865-271-853.
E-mail address: trevor.sharp@pharm.ox.ac.uk (T. Sharp).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01105-0

were housed for 7 days prior to experimentation under controlled conditions of temperature (20 °C) and humidity (50%), in a 12 h light/12 h dark cycle and provided with food and water ad libitum. All drug injections and behavioural testing occurred in the light cycle between 12:00 and 16:00 h.

2.2. Amino acid mixture

The tyrosine (and phenylalanine)-free amino acid mixture contained the following amino acids: 100 mg methionine, 200 mg threonine, 50 mg tryptophan, 350 mg lysine, 300 mg isoleucine, 450 mg leucine methyl ester HCl, 350 mg valine methyl ester HCl, in 9 ml distilled water, corrected for pH, and given as 5 ml/kg i.p. (equivalent to 1 g amino acid/kg). The mixture was administered on either one or two occasions 1 h apart. This dose regime has previously been shown to cause a reduction of regional brain tyrosine levels of the order of 50–60% within 2 h of the first injection (McTavish et al., 1999b). Control animals received an equivalent amino acid mixture supplemented with tyrosine methyl ester HCl (250 mg) and phenylalanine (250 mg).

2.3. Behavioural measurements

Rats were placed individually in Perspex cages and activity counts were recorded automatically by a row of 15 horizontal photoelectric beams connected to a computer (Columbus Instruments, Columbus, OH, USA). Animals were given 2 h to acclimatize, before being given the amino acid mixture and drug injections. Activity counts were then monitored over six consecutive 10 min periods.

Individual behaviours of each animal were also assessed by direct observation, using rating scales based on a checklist sampling method (e.g. Ainsworth et al., 1998). Behaviours rated were: sniffing (directed movement of the snout with accompanying whisker movement), locomotion (movement of all four limbs to a new position), rearing (elevation of the upper body off the floor of the cage), grooming (cleaning of the head forepaws and upper body), and intense grooming (cleaning of the hind-flank and abdomen). In the experiment examining the behavioural response to *p*-chloroamphetamine, additional ratings of reciprocal forepaw treading and flat body posture were included.

Specific behaviours were rated as present or absent during a 20 s window every minute for 5 min. This procedure was repeated every 10 min for a period of 60 min and an accumulated score was obtained (maximum score for each behaviour = 30). All ratings of behaviour were made with the observer blind to treatment.

2.4. Experimental protocol

After a 2 h habituation period, animals received either a single injection of the tyrosine-free amino acid mixture or two injections administered one hour apart (each 1 g/kg i.p.). Control animals received equivalent injections of the amino acid mixture containing tyrosine and phenylalanine. Neither of the amino acid mixtures were found to alter activity counts during the habituation period relative to saline-injected controls (data not shown).

A challenge drug was administered 1 h after the final injection of the amino acid mixture. The drug challenges were as follows: D-amphetamine (2 mg/kg s.c.), cocaine (2 or 5 mg/kg s.c.), apomorphine (0.75 or 5 mg/kg s.c.) and *p*-chloroamphetamine (2 mg/kg s.c.). Drugs were dissolved in saline and administered in a volume of 1 ml/kg.

2.5. Statistical analysis

Activity counts (mean activity counts/h) were analyzed statistically using unpaired *t*-test. Behavioural scores (mean scores/h) were analyzed by Mann–Whitney *U*-test. A P value of < 0.05 was considered significant.

2.6. Drugs and chemicals

All amino acids were L-enantiomers and used in the base form apart from tyrosine, leucine and valine which were methyl ester salts (HCl). D-amphetamine sulphate, cocaine HCl, apomorphine HCl and *p*-chloroamphetamine HCl were obtained from Sigma.

3. Results

3.1. Effect of a tyrosine-free amino acid mixture on the behavioural response to D-amphetamine

The behavioural activation induced by D-amphetamine (2 mg/kg s.c.) was significantly reduced in rats receiving the tyrosine-free amino acid mixture compared to rats receiving the same mixture supplemented with tyrosine. This effect was apparent in the measurements of both automated activity counts and behavioural scores (Fig. 1A).

Thus, the total number of activity counts induced by D-amphetamine was 35% less in rats pretreated with one injection of the tyrosine-free mixture and 50% less in rats pretreated with two injections ($P < 0.05$ versus tyrosine-containing mixture; Table 1). Moreover, the amphetamine-induced increase in behavioural scores for locomotion and rearing were significantly reduced by both one and two injections of the tyrosine-free mixture ($P < 0.05$; Fig. 1A and data not shown). The sniffing score was also reduced by the tyrosine-free mixture although this was only statistically significant for one injection (data not shown).

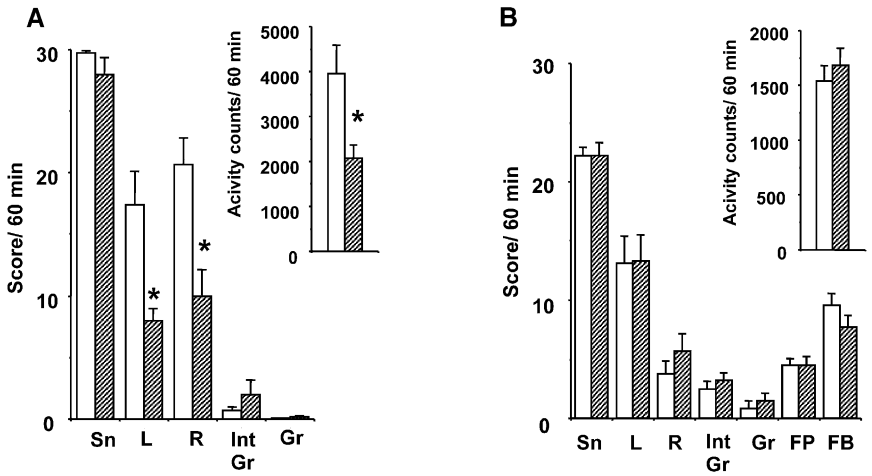

Fig. 1. Effect of a tyrosine-free amino acid mixture on behaviour induced by (A) the dopamine releasing agent d-amphetamine (2 mg/kg s.c.) and (B) the 5-HT releasing agent, *p*-chloroamphetamine (2 mg/kg s.c.). Behavioural scores and automated activity counts (insert) were accumulated over 60 min. Animals received either two tyrosine-containing mixtures (open bars) or two tyrosine-free mixtures (crossed bars) (1 g/kg i.p. separated by 1 h), with amphetamine/*p*-chloroamphetamine being administered at 1 h after the second injection. Results were analyzed statistically by either the Mann–Whitney *U*-test (behavioural ratings) or by Student's unpaired *t*-test (activity counts). Mean ± S.E.M. values are shown. $^{*}P < 0.05$, $n = 6$ in each group.

3.2. Effect of a tyrosine-free amino acid mixture on the behavioural response to cocaine

In rats pretreated with the tyrosine-free mixture (two injections), the increase in activity counts induced by 2 mg/kg s.c. cocaine was significantly less ($P < 0.05$) than in rats receiving the mixture supplemented with tyrosine (Table 1). A similar (but not statistically significant) trend was detected after 5 mg/kg s.c. cocaine (Table 1).

3.3. Effect of a tyrosine-free amino acid mixture on the behavioural response to p-chloroamphetamine

In contrast to D-amphetamine, the behavioural activation induced by the 5-HT releasing agent, *p*-chloroamphetamine (2 mg/kg s.c.) was not different in rats receiving the tyrosine-free mixture (two injections) compared to those receiving the mixture supplemented with tyrosine (Fig. 1B).

3.4. Effect of a tyrosine-free amino acid mixture on the behavioural response to apomorphine

Two injections of the tyrosine-free amino acid mixture did not affect the increase in activity induced by the direct acting dopamine agonist, apomorphine (0.75 or 5 mg/kg s.c.) (Table 1).

4. Discussion

Administration of a tyrosine (and phenylalanine)-free amino acid mixture decreases brain tyrosine by causing a

Table 1
Effect of pretreatment with a tyrosine free (T −) and tyrosine supplemented (T +) amino acid mixtures (i.p.) on the behavioural activation induced by psychostimulant drugs

Challenge drug	No. injections of amino acid mixture	Activity (counts/60 min) after T +	Activity (counts/60 min) after T −
Amphetamine (2)	1	3784 ± 380 ($n = 6$)	2450 ± 332 ($n = 6$)[a]
Amphetamine (2)	2	3950 ± 633 ($n = 6$)	2083 ± 283 ($n = 6$)[a]
Cocaine (2)	2	200 ± 17.8 ($n = 5$)	106.5 ± 22.4 ($n = 6$)[a]
Cocaine (5)	2	344.5 ± 97.6 ($n = 6$)	140.2 ± 24.4 ($n = 6$)
Apomorphine (0.75)	2	1800 ± 588 ($n = 6$)	1705 ± 500 ($n = 6$)
Apomorphine (5)	2	4583 ± 994 ($n = 6$)	4418 ± 1139 ($n = 6$)
p-Chloroamphetamine (2)	2	1710 ± 180 ($n = 6$)	1540 ± 160 ($n = 6$)
Saline	0		138 ± 33 ($n = 5$)

Data shown are activity scores summed over the 60 min period following challenge drug administration or saline. Doses in (mg/kg s.c.) are shown in brackets. Results were analyzed statistically using unpaired *t*-test. Means ± S.E.M. values as shown.

[a] $P > 0.05$.

fall in plasma tyrosine as well as blockade of tyrosine entry into the brain via the large neutral amino acid transporter (Biggio et al., 1976; Moja et al., 1996). Our recent microdialysis data show that a tyrosine-free amino acid mixture causes a fall in brain catecholamine synthesis and also prevents the release of dopamine evoked by amphetamine (McTavish et al., 1999b). Here, we report that a tyrosine-free amino acid mixture attenuates the behavioural activation induced in rats by amphetamine and cocaine. Importantly, we show that this effect is specific in that the tyrosine-free mixture used did not attenuate the stimulant effects of either the direct acting agonist apomorphine or the 5-HT releasing agent *p*-chloroamphetamine.

Compared to a tyrosine-supplemented control mixture, the tyrosine (and phenylalanine)-free amino acid mixture caused a dose-related decrease in both automated and observer-rated measures of the behavioural response to amphetamine. These data are consistent with earlier findings that amphetamine-induced hyperactivity is blocked by the tyrosine-hydroxylase inhibitor α-methyl-*p*-tyrosine (Widerlov and Levander, 1978). Although noradrenaline may play a role in some of the behavioural effects of amphetamine (e.g. Philips et al., 1982), our recent microdialysis studies indicate that the dose of tyrosine-free amino acid load used here attenuates amphetamine-induced release of dopamine but not noradrenaline (McTavish et al., 1999b). Therefore, the attenuation of amphetamine-induced behaviour is most likely to be associated with a reduction in dopamine release. This being the case, the anatomical sites of action of tyrosine depletion are likely to include nigrostriatal and mesolimbic dopamine pathways although mesocortical dopamine pathways are also a possible target.

It is unlikely that the tyrosine-free amino acid load is acting post-synaptically to block the effect of amphetamine since the mixture did not prevent the behavioural activation induced by apomorphine, even when the latter was used at a relatively high dose (5 mg/kg). Also, the tyrosine-free amino acid mixture did not reduce the behavioural effects of *p*-chloroamphetamine. The latter finding indicates that the mixture does not attenuate 5-HT release. Previously, we found that the tyrosine-depletion does not lower regional brain levels of 5-HT synthesis (McTavish et al., 1999b). Although tryptophan (the precursor of 5-HT) is also a large neutral amino acid, it is likely that with the present amino acid mixture the brain levels of tryptophan remain stable and that presynaptic 5-HT function is not influenced.

In addition to amphetamine, the tyrosine-free amino acid mixture attenuated the mild (relative to amphetamine) increase in behavioural activity seen after cocaine administration. Acute cocaine administration is well known to inhibit reuptake of dopamine and cause an increase in extracellular dopamine which is correlated with enhanced locomotor activity (Church et al.,1987; Hurd and Ungerstedt, 1989). Moreover, the stimulant effect of cocaine on locomotion is blocked by pretreatment with dopamine receptor antagonists (Ushijima et al., 1995; Le et al., 1997). Therefore, it is reasonable to assume that, as with amphetamine, the attenuation of cocaine-evoked behaviour by tyrosine depletion is due to a decrease in presynaptic dopamine function. It would be interesting to know whether tyrosine depletion strategies are able to reduce the addictive properties of cocaine and other drugs of abuse.

In summary, we show that in rats a tyrosine-free amino acid mixture attenuates the behavioural activation induced by amphetamine and cocaine. In a recent small scale study in healthy volunteers tyrosine depletion was found to decrease amphetamine-induced mood changes (McTavish et al., 1999c). Acute tyrosine depletion also reduces alcohol intake in vervet monkeys (Palmour et al., 1998). Taken together, these preclinical and clinical data support the view that tyrosine-depletion strategies have value for future research, and even treatment, of conditions where hyperactive dopaminergic pathways are implicated.

Acknowledgements

This work was supported in part by a MRC Clinical Training Fellowship (SFB McTavish).

References

Ainsworth, K., Smith, S.E., Sharp, T., 1998. Evidence that repeated administration of the antidepressant drugs fluoxetine, desipramine and tranylcypromine increases central dopamine D_2-like but not D_1-like receptor function in the rat. J. Psychopharmacol. 12, 252–257.

Biggio, G., Porceddu, M.L., Gessa, G.L., 1976. Decrease of homovanillic, dihydroxyphenylacetic acid and cyclic-adenosine-3′,5′-monophosphate content by the acute administration of an amino acid mixture lacking tyrosine and phenylalanine. J. Neurochem. 26, 1253–1255.

Church, W.H., Justice Jr., J.B., Byrd, L.D., 1987. Extracellular dopamine in rat striatum following uptake inhibition by cocaine, nomifensine and benztropine. Eur. J. Pharmacol. 139, 345–348.

Fernstrom, M.H., Fernstrom, J.D., 1995. Acute tyrosine depletion reduces tyrosine hydroxylation rate in rat central nervous system. Life Sci. 57, 97–102.

Hurd, Y.L., Ungerstedt, U., 1989. Cocaine: an in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. Synapse 3, 48–54.

Le, A.D., Tomkins, D., Higgins, G., Quan, B., Sellers, E.M., 1997. Effects of 5-HT3, D1 and D2 receptor antagonists on ethanol- and cocaine-induced locomotion. Pharmacol., Biochem. Behav. 57, 325–332.

McTavish, S.F.B., Callado, L., Cowen, P.J., Sharp, T., 1999a. Comparison of the effects of α-methyl-*p*-tyrosine and a tyrosine-free amino acid load on extracellular noradrenaline in the rat hippocampus in vivo. J. Psychopharmacol. 13, 379–384.

McTavish, S.F.B., Cowen, P.J., Sharp, T., 1999b. Effect of a tyrosine-free amino acid mixture on regional brain catecholamine synthesis and release. Psychopharmacology 141, 182–188.

McTavish, S.F.B., McPherson, M.H., Sharp, T., Cowen, P.J., 1999c. Attenuation of some subjective effects of amphetamine following tyrosine depletion. J. Psychophamacol. 13, 144–147.

Moja, E.A., Lucini, V., Benedetti, F., Lucca, A., 1996. Decrease in plasma phenylalanine and tyrosine after phenylalanine-tyrosine free amino acid solutions in man. Life Sci. 58, 2389–2395.

Palmour, R.M., Ervin, F.R., Baker, G.B., Young, S.N., 1998. An amino acid mixture deficient in phenylalanine and tyrosine reduces cerebrospinal fluid catecholamine metabolites and alcohol consumption in vervet monkeys. Psychopharmacology 136, 1–7.

Philips, S.R., Robson, A.M., Boulton, A.A., 1982. Unstimulated and amphetamine-stimulated release of endogenous noradrenaline and dopamine from rat brain in vivo. J. Neurochem. 38, 1106–1110.

Ushijima, I., Carino, M.A., Horita, A., 1995. Involvement of D1 and D2 dopamine systems in the behavioural effects of cocaine in rats. Pharmacol., Biochem. Behav. 52, 737–741.

Widerlov, E., Levander, T., 1978. Inhibition of the in vivo biosynthesis and changes of catecholamine levels in rat brain after α-methyl-para-tyrosine; time– and dose–response relationships. Naunyn-Schmeideberg's Arch. Pharmacol. 304, 111–123.