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Original

Effects of alpha-methyl-*p*-tyrosine on extracellular dopamine levels in the nucleus accumbens and the dorsal striatum of freely moving rats

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Abstract: Alpha-methyl-*p*-tyrosine (AMPT) is known to inhibit the formation of dopamine (DA) in the cytosol of dopaminergic neurons and is therefore used to study the role of the cytosolic DA pools. AMPT is usually administered systemically. In the present study, however, the effects of locally infused AMPT on the efflux of DA from the nucleus accumbens and dorsal striatum were analyzed, using *in vivo* brain microdialysis in unanesthetized rats. The administration of AMPT (100 μM, 4 h) into the nucleus accumbens reduced accumbal DA output to 30% of its baseline level. When it was infused into the dorsal striatum, however, it reduced striatal DA output to 60% of its baseline level. At first sight, these data suggest that the amount of DA available from the AMPT-sensitive pool is larger in the nucleus accumbens than in the striatum. However, this cannot be the case, as the decrease in accumbal and striatal DA efflux induced by systemic administration of AMPT (250 mg/kg given intraperitoneally) was identical. These results show that local infusion of AMPT is a valuable tool for analyzing the role of AMPT-sensitive pools within a particular brain area, but it cannot be used to compare effects across different brain structures because a fixed dose of AMPT differentially affected the nucleus accumbens and the dorsal striatum. (J. Oral Sci. 47, 185-190, 2005)

Keywords: dopamine; alpha-methyl-*p*-tyrosine; nucleus accumbens; dorsal striatum.

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Introduction

There are two sources for the release of dopamine (DA) from the terminals of dopaminergic (DAergic) neurons, namely reserpine-sensitive, vesicular storage pools that are insensitive to alpha-methyl-*p*-tyrosine (AMPT), and AMPT-sensitive, cytosolic pools that are insensitive to reserpine. Reserpine blocks, among others, vesicle monoaminergic transporter type 2 in the terminals of DAergic neurons, with the result that DA remains outside the reserpine-sensitive storage pools in the cytosol and is easily metabolized by the available monoamine oxidase. AMPT inhibits the rate-limiting enzyme tyrosine hydroxylase, with the result that it inhibits, among others, the formation of DA in the cytosol of terminals of DAergic neurons. In other words, both reserpine and AMPT can be used to reduce the amount of releasable DA. There is evidence that particular psychostimulants, such as dexamphetamine and cocaine,

differentially affect these two pools (1-3). When reserpine and/or AMPT are used to study the role of these distinct pools on drug-induced effects, they are given systemically (4-8). It is unknown whether or not these agents are also effective when administered locally. Because there is evidence that the amount of tyrosine hydroxylase varies across DAergic structures (9-11), we decided to analyze the effects of a local perfusion with AMPT on the extracellular amount of DA in both the nucleus accumbens, which is innervated primarily by DAergic mesolimbic neurons, and the dorsal striatum, which is innervated primarily by DAergic, nigrostriatal neurons. Given the remarkable differences in sensitivity to AMPT between both structures (see Results), we decided to include an additional series of experiments on the effects of systemic administration of AMPT on the extracellular levels of DA in these structures.

Materials and Methods

Animals

Male Sprague-Dawley rats (NRC Haruna, Gunma, Japan) weighing between 200 and 220 g at the start of the experiments were used. They were housed under conditions of constant room temperature (23 ± 2°C) and relative humidity (55 ± 5%), and under a 12 h light-dark cycle (lights on at 0700 h), with free access to food and water. The experiments were approved by the Animal Experimentation Committee of Nihon University School of Dentistry, and were performed in accordance with national and institutional guidelines for animal care and welfare of experimental animals, which are in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No.85 - 23, Revised 1985). All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgery

Rats were anesthetized with pentobarbital sodium (Nembutal Injection, Dainippon Pharmaceutical, Osaka, Japan, 50 mg/kg given intraperitoneally, i.p.). The anesthetized animals were placed into a stereotaxic apparatus, and a guide cannula was implanted just above the left dorsal striatum or the nucleus accumbens [dorsal striatum: anteroposterior (AP) 9.2 mm, mediolateral (ML) 3.2 mm, dorsoventral (DV) 7.2 mm; nucleus accumbens: AP 10.6 mm, ML 1.5 mm, DV 4.0 mm from the interaural line; according to the atlas of Paxinos and Watson (12)]. To avoid the ventricular system, cannulae directed at the nucleus accumbens were angled at 18° from the midsagittal plane. The length of the probe membrane (2 mm) prevented a lucid separation between the core and shell region; accordingly, the data are given per nucleus accumbens rather than per a distinct accumbal subregion. Rats were allowed to recover from surgery for 7 - 10 days before the experiments were carried out; the patency of guide cannulae (0.4 mm outer diameter, o.d.) was maintained by stainless steel inserts. Each animal was used only once.

Dialysis and neurochemical measurements

A commercially available I-shaped, removable-type dialysis probe was used (2.0-mm long cellulose membrane, 0.22 mm o.d., 50,000 molecular weight cutoff, Eicom A-I-8-02 type for the nucleus accumbens and Eicom A-I-4-02 for the dorsal striatum, Kyoto, Japan). The experiment was started by removing the stylet from the guide cannula and inserting the dialysis probe; only the dialysis membrane protruded from the tip (Fig. 1). The probe was secured to the guide cannula by a screw. Each rat was then placed into a Plexiglas box (30 × 30 × 35 cm); inlet and outlet tubes were connected to a swivel located on a counter-balanced beam to minimize discomfort. The probe was perfused at a rate of 2.0 μl/min with modified Ringer's solution (in mM: NaCl 147, KCl 4, $CaCl_2$ 1.2, $MgCl_2$ 1.1; pH 7.4), and the outflow was connected by Teflon tubing to a high-performance liquid chromatography system (Eicom). DA was separated on an Eicompak CA-5ODS column (particle size, 5 μm, 2.1 × 150 mm, Eicom) using phosphate buffer (0.1 M) containing octane-sulfonic acid (3.2 mM), ethylenediaminetetraacetic acid (0.13 mM), and 20% methanol (pH 6.0) as the mobile phase at a flow rate of 0.23 ml/min. Compounds were quantified by electrochemical detection using a glassy carbon working electrode set at +400 mV against a silver-silver chloride reference electrode (Eicom), giving a detection limit for DA of about 0.05 pg per sample. The probes had an *in vitro* recovery of approximately 12% for DA, but the reported concentrations were not adjusted for recovery *in vivo* because these estimations are inaccurate (13,14). Previous experiments in which we have used the same technique and procedure have shown that DA efflux is more or less stabilized 4 h after probe insertion, and that levels seen at that time are largely dependent upon neuronal release, as most of the release is tetrodotoxin-sensitive and Ca^{2+}-dependent (15-19). Perfusate samples were taken every 20 min for quantification of DA. When a stable baseline of DA was reached approximately 4 h after inserting the probe, AMPT (the methyl ester form, Sigma-RBI, St. Louis, USA) was perfused through the probe (100 μM, 4 h) or injected intraperitoneally (250 mg/kg), and DA efflux in the nucleus accumbens or the dorsal striatum was monitored for 4 h. To study the stability of the phenomenon, perfusates were also collected 20 h after insertion of the

probe into the dorsal striatum. The doses of AMPT used were based on the outcome of previously published studies [local administration (20,21), systemic administration (22,23)]. Baseline levels of DA were taken as the mean of the last three samples collected before drug administration.

Histology

At the end of each experiment, the rat was deeply anesthetized with pentobarbital sodium (80 mg/kg, i.p.) and perfused transcardially with 10% formaldehyde solution. The brain was removed, sectioned (50 μm), and stained with cresyl violet to permit probe location.

Statistical analysis

All values are expressed as a percentage of baseline levels. All data were analyzed with a two-way analysis of variance (ANOVA) with the factor structure or treatment and the factor time for repeated measures. Statistical significance was considered to be $P < 0.05$.

Results

Histology

Placements of the dialysis probe in the nucleus accumbens and the dorsal striatum are shown in Fig. 1.

Basal dialysate DA levels in the nucleus accumbens and the dorsal striatum

Baseline concentrations of DA in dialysates from the nucleus accumbens and the dorsal striatum 4 h after probe insertion reached relatively stable values of 2.6 ± 0.23 pg/20 min (mean ± S.E.M., $n = 15$) and 5.5 ± 0.75 pg/20 min ($n = 16$), respectively. In general, the extracellular baseline levels of striatal DA and accumbal DA in the present study did not differ from those found in our previous studies (19).

Since microdialysis experiments were carried out between days 7 and 10 after surgery, we compared the DA efflux across these days: no significant differences were observed in basal DA efflux on days 7, 8, 9, and 10 after surgery (nucleus accumbens: $F_{3,11} = 0.81$, $P = 0.51$, $n = 15$, one-way ANOVA, dorsal striatum: $F_{2,13} = 0.81$, $P = 0.47$, $n = 16$, one-way ANOVA).

Effects of AMPT perfusion on dialysate DA levels in the nucleus accumbens and the dorsal striatum

The local administration of AMPT (100 μM, 4 h) into the nucleus accumbens reduced the basal DA efflux in the nucleus accumbens by about 2.0 pg, to approximately 30% of the baseline concentration 240 min after the administration of AMPT. In the dorsal striatum, however, this treatment reduced the extracellular level of DA by about 2.4 pg, to approximately 60% of the baseline concentration 240 min after the administration of AMPT. Indeed, the effects of the local administration of AMPT (100 μM, 4 h) on accumbal and striatal DA efflux were significantly different (time × treatment: $F_{11,140} = 3.88$, $P < 0.001$, two-way ANOVA: 20-240 min after onset of the local administration of AMPT; Fig. 2).

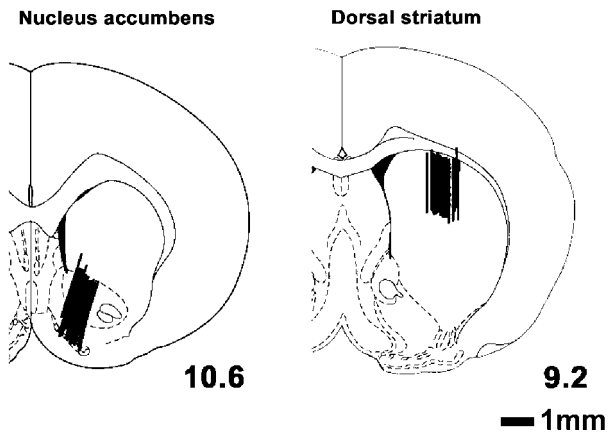

Fig. 1 Schematic illustration showing the locations of the probe in the nucleus accumbens (left) and the dorsal striatum (right). The planes are taken from the atlas of Paxinos and Watson (12), representing anterior distance (mm) from the interaural line.

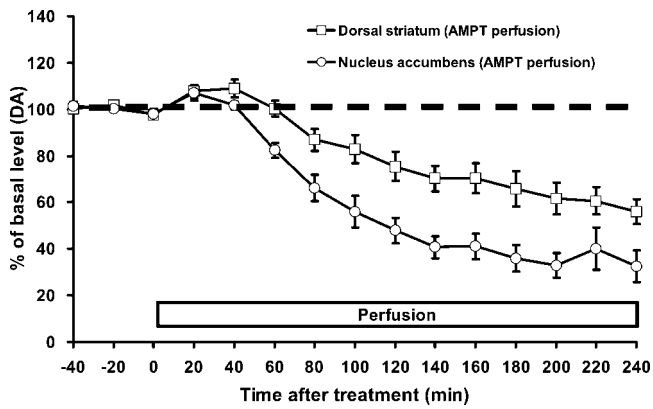

Fig. 2 Effects of local perfusion of alpha-methyl-*p*-tyrosine (AMPT; 100 μM) on basal extracellular levels of dopamine (DA) in the nucleus accumbens ($n = 7$) and the dorsal striatum ($n = 8$). The data are expressed as the mean ± S.E.M. of change (%) from baseline DA levels (ordinate) and time (min) after the onset of an infusion of AMPT (abscissa). The bar above the abscissa indicates the period of perfusion of AMPT.

Effects of systemic administration of AMPT on dialysate DA levels in the nucleus accumbens and dorsal striatum

Systemic administration of AMPT (250 mg/kg, i.p.) reduced basal DA efflux in the nucleus accumbens (n =7) and dorsal striatum (n = 8) to more or less the same amount, namely 70% of baseline levels (time × treatment: $F_{11,140}$ = 0.38, P = 0.96, two-way ANOVA: 20-240 min after AMPT administration; Fig. 3). The differences in the sensitivity to local and systemic administration of AMPT of striatal DA levels were still present 20 h after probe insertion (time × treatment: $F_{8,80}$ = 26.6, P < 0.001, two-way ANOVA: 20-180 min after onset of AMPT administration; Fig. 4).

Discussion

The present study clearly reveals that local administration of AMPT can be used to study the role of the DAergic, cytosolic pools on the amount of DA released from DAergic terminals into the synaptic cleft, since the locally infused AMPT, which is known to inhibit the formation of DA in the cytosolic pool (see Introduction), significantly reduced both accumbal and striatal DA levels. Accordingly, this technique can also be used to assess the role of these pools on the effects induced by psychostimulants such as dexamphetamine and cocaine.

Basal DA output in the nucleus accumbens (2.6 ± 0.23 pg/20 min) was far lower than that of the dorsal striatum (5.5 ± 0.75 pg/20 min). The absolute reduction of DA induced by the local administration of AMPT in the nucleus accumbens (approximately 2.0 pg; basal level 2.8 pg, after AMPT treatment 0.8 pg) and the dorsal striatum (approximately 2.4 pg; basal level 5.7 pg, after AMPT treatment 3.3 pg) appeared to be more or less equal. However, as Di Chiara et al. (24) have pointed out, absolute changes should be corrected for basal dialysate levels and expressed as relative (%) changes when carrying out comparisons between areas of differing basal DA output.

The present study shows that both local and systemic administration of AMPT significantly reduces the DA efflux from the dorsal striatum and the nucleus accumbens (Figs. 2 and 3). This effect remained stable with time, as shown by the finding that both local and systemic administration of AMPT given 24 h after probe insertion still reduced the efflux of DA from the striatum (Fig. 4). Whether this also holds true for the nucleus accumbens remains to be investigated.

Remarkably, the effects of locally infused AMPT were relatively far greater in the nucleus accumbens than in the dorsal striatum, as was indicated by the relative change. At first sight, these data suggest that the number of DAergic, cytosolic pools and/or the amount of DA per cytosolic pool is far greater in the nucleus accumbens than in the dorsal striatum. However, this cannot be the case, because when AMPT was given systemically the rate of reduction of extracellular DA in the nucleus accumbens was identical to that seen in the dorsal striatum (Fig. 3). Of course, it must be realized that the microdialysis technique has

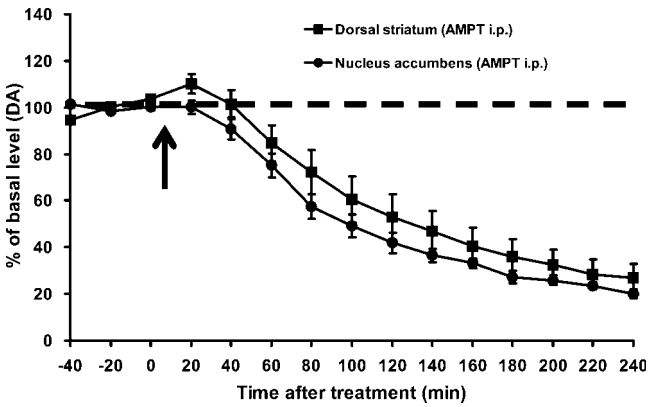

Fig. 3 Effects of systemic administration of AMPT (250 mg/kg i.p.) on basal extracellular levels of DA in the nucleus accumbens (n = 8) or the dorsal striatum (n = 7). The data are expressed as the mean ± S.E.M. of change (%) from baseline DA levels (ordinate) and time (min) after the systemic administration of AMPT (abscissa).

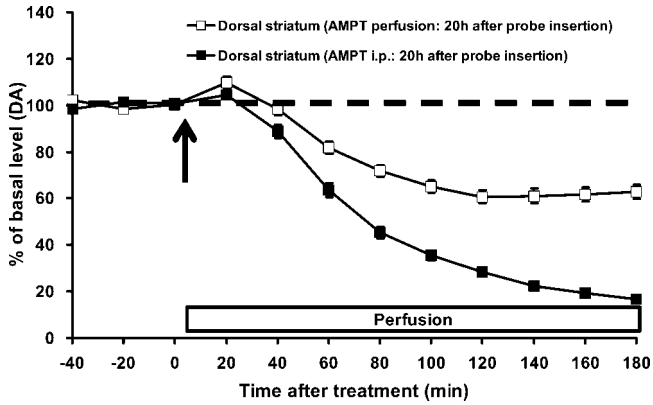

Fig. 4 Effects of local perfusion (100 μM; n = 8) and systemic administration (250 mg/kg i.p.; n = 4) of AMPT on basal extracellular levels of DA in the dorsal striatum. The administrations of AMPT were carried out 20 h after probe insertion. The data are expressed as the mean ± S.E.M. of change (%) from baseline DA levels (ordinate) and time (min) after the onset of AMPT treatments (abscissa). The bar above the abscissa indicates the period of perfusion of AMPT.

clear-cut limitations in the sense that subtle differences between subregions that are differentially innervated by nigrostriatal and mesolimbic neurons within the nucleus accumbens or the dorsal striatum cannot be detected with this method. In other words, the present study does not allow the conclusion that there are no differences in terms of the number of DAergic, cytosolic pools and/or the amount of DA per cytosolic pool between the nucleus accumbens and the dorsal striatum.

The finding of differences in DA output between the nucleus accumbens and the dorsal striatum after local administration of AMPT can be explained simply by differences in the density of nerve terminals, blood flow, dimensions of the perfused tissue, and/or diffusion across the brain region under study. Thus, the present study evidently shows that the local administration of AMPT is a valuable tool for analyzing the role of AMPT-sensitive pools on drug-induced effects within a particular brain structure (20,21), but it cannot be used to compare effects across different brain structures.

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References

1. Butcher SP, Fairbrother IS, Kelly JS, Arbuthnott GW (1988) Amphetamine-induced dopamine release in the rat striatum: an in vivo microdialysis study. J Neurochem 50, 346-355
2. Cadoni C, Pinna A, Russi G, Consolo S, Di Chiara G (1995) Role of vesicular dopamine in the *in vivo* stimulation of striatal dopamine transmission by amphetamine: evidence from microdialysis and Fos immunohistochemistry. Neuroscience 65, 1027-1039
3. Florin SM, Kuczenski R, Segal DS (1995) Effects of reserpine on extracellular caudate dopamine and hippocampus norepinephrine responses to amphetamine and cocaine: mechanistic and behavioral considerations. J Pharmacol Exp Ther 274, 231-241
4. Biggs CS, Fowler LJ, Whitton PS, Starr MS (1996) NMDA receptor antagonists increase the release of dopamine in the substantia nigra of reserpine-treated rats. Eur J Pharmacol 299, 83-91
5. Biggs CS, Fisher A, Starr MS (1998) The antiparkinsonian drug budipine stimulates the activity of aromatic L-amino acid decarboxylase and enhances L-DOPA-induced dopamine release in rat substantia nigra. Synapse 30, 309-317
6. Butcher SP, Varro A, Kelly JS, Dockray GJ (1989) In vivo studies on the enhancement of cholecystokinin release in the rat striatum by dopamine depletion. Brain Res 505, 119-122
7. Fairbrother IS, Arbuthnott GW, Kelly JS, Butcher SP (1990) In vivo mechanisms underlying dopamine release from rat nigrostriatal terminals: I. Studies using veratrine and ouabain. J Neurochem 54, 1834-1843
8. Fairbrother IS, Arbuthnott GW, Kelly JS, Butcher SP (1990) In vivo mechanisms underlying dopamine release from rat nigrostriatal terminals: II. Studies using potassium and tyramine. J Neurochem 54, 1844-1851
9. Brauer K, Hausser M, Hartig W, Arendt T (2000) The core-shell dichotomy of nucleus accumbens in the rhesus monkey as revealed by double-immunofluorescence and morphology of cholinergic interneurons. Brain Res 858, 151-162
10. Raisman-Vozari R, Hirsch E, Javoy-Agid F, Vassort C, Savasta M, Feuerstein C, Thibault J, Agid Y (1991) Quantitative autoradiography of tyrosine hydroxylase immunoreactivity in the rat brain. J Neurochem 57, 1212-1222
11. Zahm DS (1992) An electron microscopic morphometric comparison of tyrosine hydroxylase immunoreactive innervation in the neostriatum and the nucleus accumbens core and shell. Brain Res 575, 341-346
12. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. 2nd ed, Academic press, New York
13. Benveniste H, Hansen AJ, Ottosen NS (1989) Determination of brain interstitial concentrations by microdialysis. J Neurochem 52, 1741-1750
14. Lindefors N, Amberg G, Ungerstedt U (1989) Intracerebral microdialysis: I. Experimental studies of diffusion kinetics. J Pharmacol Methods 22, 141-156
15. Takada K, Murai T, Kanayama T, Koshikawa N (1993) Effects of midazolam and flunitrazepam on

the release of dopamine from rat striatum measured by *in vivo* microdialysis. Br J Anaesth 70, 181-185
16. Tomiyama K, Noguchi M, Koshikawa N, Kobayashi M (1993) YM-09151-2 but not *l*-sulpiride induces transient dopamine release in rat striatum via a tetrodotoxin-insensitive mechanism. J Neurochem 60, 1690-1695
17. Murai T, Koshikawa N, Kanayama T, Takada K, Tomiyama K, Kobayashi M (1994) Opposite effects of midazolam and β-carboline-3-carboxylate ethyl ester on the release of dopamine from rat nucleus accumbens measured by in vivo microdialysis. Eur J Pharmacol 261, 65-71
18. Tomiyama K, Koshikawa N, Funada K, Oka K, Kobayashi M (1995) *In vivo* microdialysis evidence for transient dopamine release by benzazepines in rat striatum. J Neurochem 65, 2790-2795
19. Fusa K, Saigusa T, Koshikawa N, Cools AR (2002) Tyrosine-induced release of dopamine is under inhibitory control of presynaptic dopamine D2 and, probably, D3 receptors in the dorsal striatum, but not in the nucleus accumbens. Eur J Pharmacol 448, 143-150
20. Saigusa T, Tuinstra T, Koshikawa N, Cools AR (1999) High and low responders to novelty: effects of a catecholamine synthesis inhibitor on novelty-induced changes in behaviour and release of accumbal dopamine. Neuroscience 88, 1153-1163
21. Tuinstra T, Cools AR (2000) Newly synthesized dopamine in the nucleus accumbens is regulated by β-adrenergic, but not α-adrenergic, receptors. Neuroscience 98, 743-747
22. Scheel-Krüger J (1971) Comparative studies of various amphetamine analogues demonstrating different interactions with the metabolism of the catecholamines in the brain. Eur J Pharmacol 14, 47-59
23. Fusa K, Takahashi I, Watanabe S, Aono Y, Ikeda H, Saigusa T, Nagase H, Suzuki T, Koshikawa N, Cools AR (2005) The non-peptidic delta opioid receptor agonist TAN-67 enhances dopamine efflux in the nucleus accumbens of freely moving rats via a mechanism that involves both glutamate and free radicals. Neuroscience 130, 745-755
24. Di Chiara G, Tanda G, Carboni E (1996) Estimation of in-vivo neurotransmitter release by brain microdialysis: the issue of validity. Behav Pharmacol 7, 640-657