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Characterization of the phencyclidine-induced increase in prefrontal cortical dopamine metabolism in the rat

Asami Umino, [2]Kiyohisa Takahashi & [1]Toru Nishikawa

Department of Mental Disorder Research, National Institute of Neuroscience, NCNP, 4-1-1, Ogawa-Higashi, Kodaira-shi, Tokyo 187, Japan

1 We have investigated the effects of a schizophrenomimetic drug phencyclidine (PCP) and N-methyl-D-aspartate (NMDA)-related agents alone or in combination on dopamine metabolism in the medial prefrontal cortex and striatum of the rats by measuring the tissue concentrations of dopamine and its metabolite, 3,4-dihyroxyphenylacetic acid (DOPAC), and the rate of dopamine disappearance (dopamine utilization) after its synthesis inhibition.

2 Systemic injection of PCP and selective, non-competitive, NMDA antagonists caused an increase of both tissue concentrations of DOPAC and dopamine utilization in the prefrontal cortex but not in the striatum. The PCP-induced augmentation of cortical dopamine metabolism was not influenced by selective lesion of ascending noradrenergic neurones.

3 Intra-prefrontal cortical infusion of PCP or selective competitive or non-competitive antagonists of the NMDA receptor mimicked the ability of systemic PCP injection to enhance DOPAC levels and dopamine utilization in the prefrontal cortex. However, an NMDA antagonist injected into the cell body area of the mesocortical dopaminergic neurones failed to affect dopamine metabolism in the prefrontal cortex.

4 The increasing effects of PCP and selective NMDA antagonists on cortical dopamine utilization were not additive, although a dopamine receptor antagonist, haloperidol, still accelerated the disappearance of dopamine, even in the presence of PCP.

5 Intra-cortical or intra-ventricular infusion of NMDA or D-alanine but not L-alanine, attenuated the ability of systemic PCP administration to facilitate prefrontal dopamine utilization.

6 These data suggest that PCP might activate prefrontal cortical dopaminergic neurones, at least in part, by blocking the NMDA receptor in the prefrontal cortex which participates in a tonic inhibitory control of the mesoprefrontal dopaminergic projections.

Keywords: 3,4-Dihydroxyphenylacetic acid; dopamine; dopamine utilization; excitatory amino acid; N-methyl-D-aspartate receptor; noradrenaline; phencyclidine; prefrontal cortex; striatum; strychnine-insensitive glycine site

Introduction

It has been well documented that a powerful schizophrenomimetic drug, phencyclidine (1-phenylcyclohexylpiperazine: PCP), preferentially activates cortical rather than striatal dopamine metabolism in the rodent brain *in vivo* (Bowers & Hoffman, 1984; Deutch *et al*., 1987; Rao *et al*., 1990; Nishijima *et al*., 1996). This regional selectivity is particularly interesting because the cortical dopaminergic neurones have been considered to be a therapeutic target of antiphychotic drugs (Scatton *et al*., 1975; Le Moal & Simon, 1991). Recent PET studies support the view that cognitive disturbances of schizophrenic patients might be associated with prefrontal dopaminergic dysregulation (Dolan *et al*., 1995; Okubo *et al*., 1997). Furthermore, a phencyclidine-like anaesthetic drug, ketamine, has been shown to cause in healthy volunteers a psychotic symptom, conceptual disorganization, that is significantly related to prefrontal activation (Breier *et al*., 1997).

However, the exact nature of the PCP-induced activation of cortical dopamine metabolism is still unclear. Because PCP acts as a noradrenaline (NA) as well as a dopamine uptake inhibitor (Garey & Heath, 1976; Smith *et al*., 1977) and because noradrenergic innervation is very sparse in the striatum compared to that in the cortical areas (Versteeg *et al*., 1976), PCP-induced overactivation of noradrenergic neurotransmission could underlie the selective cortical facilitation of dopamine metabolism. Alternatively, PCP might act via its potent blockade of the ion channel associated with the N-methyl-D-aspartate (NMDA) receptor (Kemp *et al*., 1987). In fact, selective, competitive antagonists for the NMDA receptor accelerate both dopamine metabolism (Hata *et al*., 1990; Nishikawa *et al*., 1991) and extracellular dopamine release (Nishijima *et al*., 1994) in the rat medial prefrontal cortex. However, this possibility is challenged by the observation that dopamine metabolism in the pyriform cortex has been shown to be augmented by PCP but not by competitive NMDA antagonists (Rao *et al*., 1990).

Recently, our data obtained from the comparison between prefrontal and striatal extracellular release of dopamine and its metabolites (Nishijima *et al*., 1996) have indicated that the augmented dopamine release seen following systemic administration of PCP might be caused by at least two mechanisms: an increase in electrical activity of the dopaminergic neurones and uptake inhibition of basally-released dopamine without changes in their firing rate. The increased impulse traffic appears to be due to NMDA antagonism by PCP because selective NMDA antagonists such as dizocilpine (MK-801; Kashiwa *et al*., 1995) and cis-4-phosphonomethyl-2-piperidine carboxylic acid (CGS19755; Nishijima *et al*., 1994) produce a tetrodotoxin-sensitive enhancement of extracellular dopamine

[1] Author for correspondence.
[2] Present address: National Center Hospital for Mental, Nervous and Muscular Disorders, NCNP, 4-1-1, Ogawa-Higashi, Kodaira-shi, Tokyo 187, Japan.

release in the medial prefrontal cortex of the rat. To assess further the mechanism by which PCP may enhance cortical dopaminergic neurotransmission, we have investigated the effects of PCP and other NMDA-related agents alone or in combination on dopamine metabolism in the medial prefrontal cortex and striatum of rats. We have also examined the influence of a selective lesion of ascending noradrenergic neurones on the ability of PCP to augment prefrontal dopamine metabolism.

Methods

Animals

Male Wistar albino rats (ST strain, Shizuoka Laboratory Animals, Japan) weighing 200–250 g were used. The animals were housed at 22±0.5°C and at 50% humidity under a controlled dark-light cycle (light: 08 h 00 min–20 h 00 min) and had free access to food and water. The present animal experiments were performed in strict accordance with the guidance of the National Institute of Neuroscience, National Center of Neurology and Psychiatry, and were approved by the Animal Investigation Committee of the Institute.

Intraventricular and intracranial injection of drugs

Bilateral injection of drugs into the medial prefrontal cortex, ventral tegmental area (VTA) or lateral ventricle of conscious rats was performed via indwelling cannulae as described previously (Nishikawa & Scatton, 1983). Briefly, stainless-steel guide cannulae (external diameter, 0.55 mm) were implanted stereotaxically under pentobarbitone (40 mg kg^{-1}, i.p.) anaesthesia 5–7 days before the experiments. On the day of the experiment, the mandrel was replaced by an injection cannula (external diameter, 0.33 mm) which extended 2 (prefrontal cortex and lateral ventricle) or 3 mm (VTA) below the guide cannula. The following coordinates from the atlas of König & Klippel (1963) were used: medial prefrontal cortex, A +10.5, V +6.3, L±0.8; VTA, A +2.2, V −2.0, L±0.85; lateral ventricle, A −0.8, V +2.0, L±1.5 (this latter used the atlas of Paxinos & Watson, 1986). The injection cannula was connected to a motor-driven 10 μl Hamilton syringe by polyethylene tubing. Drugs were infused in a volume of 2 μl for 4 min for the prefrontal cortex and VTA and 5 μl for 4 min for the cerebral ventricle. The injection cannulae were left in place for a further 2 min (cortex and VTA) or 5 min (lateral ventricle) before removal. In the drug interaction experiments, NMDA, D- or L-alanine was locally injected 5 min after intracranial infusion of 3-(±)-(2-carboxypiperazin-4-yl)-propyl-1-phosphonate (CPP) or immediately after i.p. injection of PCP. Control animals received the respective vehicle instead.

At the end of the experimental session, the location of the sites of injection cannulae was verified macroscopically in every case on the fresh brain slices for dissection of discrete areas. To obtain information on the likely extent of diffusion of the excitatory amino acid agents when locally applied into the medial prefrontal cortex, [^{3}H]-CPP (NEN, 24.3 Ci mmol^{-1}, 2 μCi 2 μl^{-1} per side) was infused into the medial prefrontal cortex of conscious rats under the same conditions used for the local infusion experiments and the animals were killed by cervical dislocation 30 min later. The frozen coronal brain sections (20 μm thick) were prepared at −20°C with a microtome-cryostat and thaw-mounted onto microscope slides. Autoradiographs were prepared by apposing the slide-mounted tissue sections on ^{3}H-sensitive film (LKB) for 4 weeks at −80°C. The radioactivity of [^{3}H]-CPP was found to be distributed throughout the whole medial prefrontal cortex but not the adjacent brain portions such as the striatum or nucleus accumbens. The extent of diffusion of D- and L-alanine in the lateral ventricle was also estimated by infusing methylene blue dissolved in the drug solution into the ventricle.

Chemical lesion

Bilateral degeneration of the ascending noradrenergic pathways was achieved by injection of 6-hydroxydopamine (6-OHDA, 2 μg in 1 μl for 3 min) into the pedunculus cerebellaris superior (coordinates: A −8.0, V −6.9, L ±1.4, Paxinos & Watson, 1986) under pentobarbitone (40 mg kg^{-1}, i.p.) anaesthesia 14 days before the experiments. The extent of degeneration of catecholaminergic neurones was verified by measuring NA levels in the prefrontal cortex.

Brain dissection

For the experiments with methyl-p-tyrosine methyl ester (α-MT), the excitatory amino acid receptor-related agents were applied systemically or locally 5 min after i.p. administration of α-MT. The animals were killed by cervical dislocation 30 or 60 min after application of these agents (the first injection in the drug interaction experiments). Then, the medial prefrontal cortex (dopamine-rich portion according to Björklund & Lindvall, 1984) (+4.5 to +3.0), nucleus accumbens (+2.7 to +1.2) and striatum (+2.0 to +0.0) were dissected out in the cold, frozen immediately on dry ice and stored at −80°C until biochemical analysis.

Quantitative analysis of dopamine, NA and dopamine metabolites

Two different parameters of dopamine metabolism were evaluated; (1) dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) levels (DOPAC/dopamine ratios were also calculated); (2) dopamine utilization as estimated by measuring the rate of dopamine disappearance for 35 min after i.p. injection of the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine methyl ester hydrochloride (α-MT, 250 mg kg^{-1}; Sharman, 1981). NA utilization was simultaneously assessed in the latter experiments by measuring the rate of disappearance of NA (Sharman, 1981). For the experiments on catecholamine utilization, various drugs were injected 5 min after α-MT administration according to our previous study (Nishikawa *et al*., 1986), because the inhibition of tyrosine hydroxylase activity was confirmed by the observation that there was a significant reduction in dopamine levels in the prefrontal cortex 5 min after i.p. injection of α-MT (dopamine in ng mg^{-1} protein was: controls 1.20±0.09, α-MT 0.96±0.07*, *$P<0.05$, $n=8-10$ (Student's t test)).

NA, dopamine and DOPAC in the brain tissues were determined by reverse-phase high-performance liquid chromatography (h.p.l.c.) with electrochemical detection (ECD), as described elsewhere (Tanii *et al*., 1990), with minor modifications. Briefly, after the addition of 3,4-dihydroxyhydrocinnamic acid as an internal standard, brain tissues were homogenized in 0.1 M perchloric acid containing 2 mM EDTA and 4 mM sodium pyrosulphate, and the homogenates were centrifuged at 8,800 × g for 20 min at 4°C. The resultant supernatant layer (250 μl) was mixed with 1 M sodium acetate (50 μl) to reach an optimum pH and was stored at −80°C until

use. Monoamines and their metabolites were separated on a stainless-steel column (4 × 250 mm) filled with 5 μm C18 (octadesil) with 0.1 M acetate-citrate buffer (pH 4.1) containing 15% methanol, 0.7 mM 1-octanesulphonic acid and 0.1 mM EDTA pumped at 0.6 ml min^{-1}. ECD was achieved with a carbon graphite working electrode set at +0.7 V. Protein content was measured according to the method of Lowry *et al.* (1951).

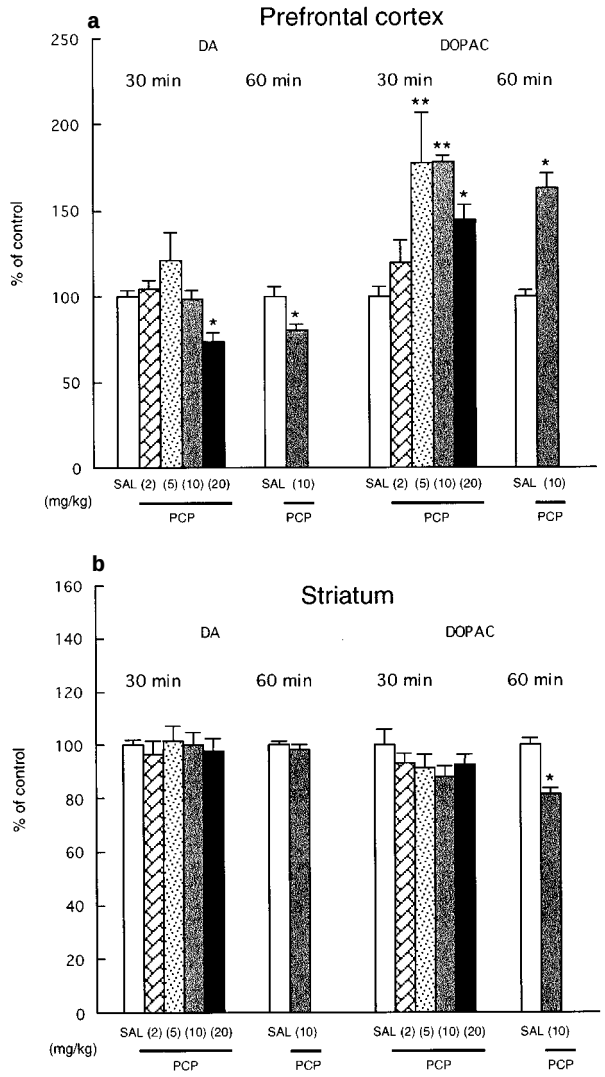

Figure 1 Effects of systemic administration of PCP on tissue concentrations of dopamine (DA) and DOPAC in the medial prefrontal cortex (a) and striatum (b) of the rat. Animals received PCP (2–20 mg kg^{-1}) or saline 30 or 60 min before being killed. Results are the means with s.e.mean of data obtained from 5–9 animals and are expressed as a percentage of respective control values which were (ng mg^{-1} protein): medial prefrontal cortex, 30 min, DA 0.93±0.04, DOPAC 0.58±0.03, $n=5$; 60 min, DA 1.06±0.04, DOPAC 0.47±0.02, $n=9$; striatum, 30 min, DA 119±2, DOPAC 29.8±1.7, $n=5$; 60 min, DA 165±3, DOPAC 34.8±0.9, $n=9$. $^{*}P<0.05$, $^{**}P<0.01$ compared to saline-injected controls. Statistical analysis was performed by two-tailed Student's t test (prefrontal cortex and striatum, 60 min), the Kruskal-Wallis test followed by the Williams-Wilcoxon test (heterogeneous variance: prefrontal cortex, 30 min), or ANOVA followed by Duncan's test (homogeneous variance: striatum, 30 min) [(a) prefrontal cortex: DA (30 min), $\chi^2(4)=12.325$, $P<0.05$; DA (60 min), $t=3.495$, $P<0.01$; DOPAC (30 min), $\chi^2(4)=14.356$, $P<0.01$; DOPAC (60 min), $t=6.344$, $P<0.01$, (b) striatum: DA (30 min), F (4, 16) = 0.957, NS (no significant difference); DA (60 min), $t=0.699$, NS; DOPAC (30 min), $F(4, 16) = 0.180$, NS; DOPAC (60 min), $t=5.480$, $P<0.01$].

Drugs

PCP hydrochloride and thienyl phencyclidine (N-1-2-thienyl]-cyclohexyl)-piperidine: TCP) hydrochloride were kindly synthesized and donated by Sumitomo Pharmaceutical, Co. Ltd. (Osaka, Japan). For local injection, these two compounds along with DL-2-amino-5-phosphonopentanoate (DL-AP5), DL-2-amino-7-phosphonoheptanoate (DL-AP7), CPP, γ-glutamylamino-methyl sulphonate (GAMS), L-glutamic acid diethyl ester (GDEE), NMDA (all from Tocris Cookson), and D- and L-alanine (Nakalai Tesque) were dissolved in phosphate-buffered saline (PBS, pH 7.4). PCP hydrochloride, TCP hydrochloride, MK-801 (Research Biochemicals Inc.), and methamphetamine (MetAMPH) hydrochloride (Dainippon Pharmaceutical, Co. Ltd.) were dissolved in saline (154 mM) for intraperitoneal (i.p.) injection. Ampoule solution of haloperidol (Hal) and its vehicle (Dainippon Pharmaceutical, Co. Ltd.) were diluted by 154 mM saline or PBS to appropriate concentrations for relevant study. For chemical lesion experiments, 6-OHDA hydrochloride (Sigma) was dissolved in physiological saline containing 0.02% w/v ascorbic acid. α-MT (250 mg kg^{-1}; Sigma) was dissolved in distilled water and injected i.p. Except for α-MT (see above), doses of these agents always refer to the free bases.

Statistics

For comparison between the two groups, the statistical significance of the data was evaluated by two-tailed Student's t test. Statistical differences between more than three groups were estimated by one-way analysis of variance (ANOVA; homogeneous variance) followed by a multiple comparison test, Duncan's test (the same number of animals in each group) or Scheffé's test (the different number of animals in each group), or the Kruskal-Wallis test (heterogeneous variance) followed by a multiple comparison test (the Williams-Wilcoxon test).

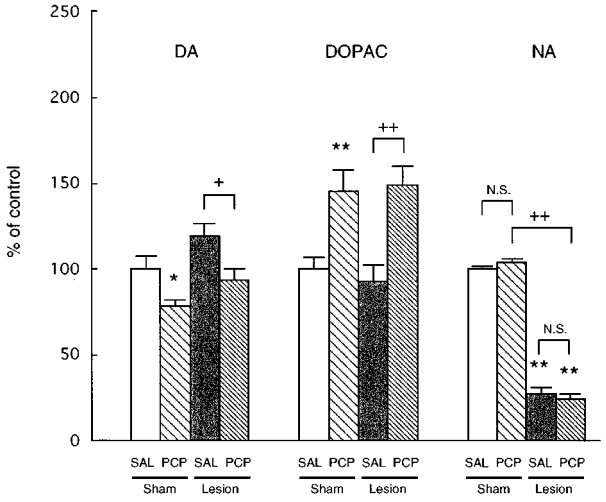

Figure 2 Effects of destruction of ascending noradrenergic neurones on the ability of PCP to increase dopamine (DA) metabolism in the medial prefrontal cortex of the rat. Fourteen days following bilateral infusion of 6-OHDA into the superior cerebellar peduncle, animals received PCP (10 mg kg^{-1}, i.p.) or saline 30 min before being killed. Results are as presented in Figure 1 ($n=6$) and the respective control values were (ng mg^{-1} protein): DA 1.38±0.10, DOPAC 0.59±0.04, NA 3.54±0.07. $^{*}P<0.05$, $^{**}P<0.01$ vs saline-injected sham-operated controls. $^{+}P<0.05$, $^{++}P<0.01$ between the two groups linked with a solid line. Statistical analysis was performed by use of ANOVA followed by Duncan's test [DA, $F(3, 20)=6.841$, $P<0.01$; DOPAC, $F(3, 20)=8.626$, $P<0.01$; NA, $F(3, 20)=278.862$, $P<0.01$].

Results

Systemic PCP: effects on dopamine and DOPAC contents of medial prefrontal cortex and striatum with or without destruction of ascending noradrenergic neurones

As shown in Figure 1a, PCP (2–20 mg kg^{-1}, i.p.) caused a marked increase in DOPAC concentration with no change or a small but significant decrease in dopamine levels of the medial prefrontal cortex at 30 and 60 min post-injection. In contrast, PCP elicited no change or a slight diminution in DOPAC concentration without affecting dopamine levels in the striatum (Figure 1b).

Bilateral infusion of 6-OHDA into the pedunculus cerebellaris superior resulted in a large reduction of cortical NA levels to 27% of sham-operated controls but no change in cortical dopamine concentrtions 14 days after the infusion (Figure 2). The selective destruction of ascending noradrenergic pathways failed to alter the ability of PCP either to diminish the dopamine concentrations or to augment that of DOPAC in the cortical region (Figure 2).

Systemic TCP or MK-801: effects on dopamine and DOPAC contents of medial prefrontal cortex and striatum

There was a dose-dependent and prominent increase in cortical DOPAC without changes in cortical dopamine levels 30 min after i.p. injection of selective and non-competitive NMDA antagonists, TCP and MK801 (Collingridge & Lester, 1989; Table 1a). However, in the striatum, these drugs did not alter the concentrations of dopamine or its metabolite at 30 min post-injection (Table 1a). Similar results were obtained 60 min after systemic administration of these NMDA antagonists (Table 1b).

Table 1 Effects of systemic injection of MK-801 and TCP on dopamine (DA) and DOPAC contents in the rat prefrontal cortex and striatum at 30 or 60 min post-injection

	Prefrontal cortex		*Striatum*	
Injection (mg kg^{-1})	*DA*	*DOPAC*	*DA*	*DOPAC*
	(ng mg^{-1} protein)		(ng mg^{-1} protein)	
a 30 min				
Saline	0.96±0.07	0.65±0.04	121±3	26±1
MK-801 (0.25)	0.94±0.02	0.94±0.10	118±4	26±1
MK-801 (1.25)	0.88±0.06	1.37±0.11**	117±2	29±1
TCP (3)	1.00±0.10	0.84±0.13	119±4	25±1
TCP (10)	0.99±0.05	1.32±0.05**	117±3	27±1
b 60 min				
Saline	1.23±0.08	0.58±0.03	120±3	26±1
MK-801 (0.25)	1.32±0.08	0.98±0.09	124±4	28±1
MK-801 (1.25)	1.20±0.08	1.38±0.09**	123±3	29±1
TCP (3)	1.43±0.10	0.82±0.10	123±6	27±1
TCP (10)	1.10±0.07	1.01±0.09**	125±3	28±1

Results are means with s.e.mean of data obtained from 4–8 animals per group (ng mg^{-1} protein). **$P<0.01$ compared to respective saline-injected controls. Statistical analysis was performed by use of ANOVA followed by Scheffé's test [(a) 30 min: prefrontal DA, $F(4, 28)=0.532$, NS (no significant difference); prefrontal DOPAC, $F(4, 29)=14.456$, $P<0.01$; striatal DA, $F(4, 29)=0.452$, NS; striatal DOPAC, $F(4, 29)=2.497$, NS, (b) 60 min; prefrontal DA, $F(4, 27)=1.906$, NS; prefrontal DOPAC, $F(4, 27)=13.472$, $P<0.01$; striatal DA, $F(4, 28)=0.353$, NS; striatal DOPAC, $F(4, 28)=1.184$, NS].

Table 2 Effects of local infusion of DL-AP7, GAMS or DL-AP5 into the prefrontal cortex or the VTA on the levels of dopamine (DA) and DOPAC in the prefrontal cortex or the nucleus accumbens of the rat

	Prefrontal cortex		*Nucleus accumbens*	
Infusion (nmol/rat)	*DA*	*DOPAC*	*DA*	*DOPAC*
	(ng mg^{-1} protein)		(ng mg^{-1} protein)	
a DL-AP7 and GAMS				
Intra-prefrontal cortex				
PBS	1.04±0.04	0.64±0.04	–	–
DL-AP7 (102)	0.78±0.04	1.07±0.10**	–	–
GAMS (102)	0.94±0.07	0.71±0.04	–	–
GAMS (204)	1.04±0.10	0.76±0.09	–	–
b DL-AP5				
Intra-prefrontal cortex				
PBS	1.06±0.04	0.58±0.04	–	–
DL-AP5 (102)	1.09±0.05	0.92±0.04**	–	–
Intra-VTA				
PBS	1.15±0.09	0.41±0.04	85±4	27±1
DL-AP5 (10)	1.27±0.09	0.44±0.04	85±2	29±2
DL-AP5 (102)	1.34±0.06	0.44±0.03	93±5	40±3**

DL-AP7, GAMS, DL-AP5 or PBS was injected into the bilateral medial prefrontal cortex or VTA and animals were killed 30 min thereafter. Results are as presented in Table 1 ($n=6–9$, ng mg^{-1} protein). **$P<0.01$ compared to respective PBS-infused controls. ANOVA followed by Duncan's test or two-tailed Student's t test [(a) DL-AP7 and GAMs into the prefrontal cortex: prefrontal DA, $F(3, 20)=2.083$, NS; prefrontal DOPAC, $F(3, 20)=6.749$, $P<0.01$, (b) DL-AP5 into the prefrontal cortex: prefrontal DA, $t=0.411$, NS; prefrontal DOPAC, $t=6.644$, $P<0.01$; DL-AP5 into the VTA: prefrontal DA, $F(2, 15)=0.978$, NS; prefrontal DOPAC, $F(2, 15)=0.262$, NS; accumbal DA, $F(2, 15)=1.452$, NS; accumbal DOPAC, $F(2, 15)=10.788$, $P<0.01$].

Intra-cortical injection of AP7 or GAMS: effects on cortical dopamine and DOPAC concentrations

Table 2a indicates that a competitive NMDA antagonist, DL-AP7, induced a significant increase in prefrontal cortical DOPAC while a non-NMDA receptor antagonist, GAMS, failed to affect dopamine metabolism in the prefrontal cortex.

Effects of intra-VTA or intra-cortical injection DL-AP5 on dopamine and DOPAC levels in the medial prefrontal cortex or nucleus accumbens

As shown in Table 2b, local injection of DL-AP5 (102 nmol/rat) into the VTA, which contains the cell bodies of prefrontal dopaminergic projections, did not change the dopamine and DOPAC content in the medical prefrontal cortex whereas, in accordance with our previous findings (Hata *et al*., 1990), intra-prefrontal cortex infusion of the drug elevated cortical levels of DOPAC. The lack of effect of intra-VTA application does not seem to be due to an inadequate amount or extent of diffusion of the NMDA antagonist, because this local infusion caused a significant increase in DOPAC levels in the nucleus accumbens which is another projection area of those dopaminergic neurons originating from the VTA (Table 2b).

Effects of systemic administration of PCP, MK-801, TCP, MetAMPH or Hal on dopamine or NA utilization in the medial prefrontal cortex and striatum

Intraperitoneal administration of PCP (Figure 3a), MK-801 (Figure 3b) or TCP (Figure 3c) produced a dose-related facilitation of dopamine utilization in the prefrontal cortex without affecting prefrontal NA utilization and striatal dopamine utilization at 30 min post-injection. In contrast, systemic injection of the dopamine receptor antagonist, Hal, accelerated both prefrontal and striatal dopamine disappearance but did not modify the prefrontal NA disappearance (Figure 3c). As shown in Figure 3c, an indirect dopamine and NA agonist, MetAMPH (Creese, 1983), decelerated dopamine and NA utilization in the prefrontal cortex and/or striatum.

Effects of local infusion of NMDA antagonists and a non-NMDA antagonist into the medial prefrontal cortex on dopamine and NA utilization in the cortical region

Intra-prefrontal injection of PCP (10–102 nmol/rat) or a selective non-competitive NMDA antagonist, TCP (102 nmol/rat) facilitated dopamine utilization in the medial prefrontal cortex (Table 3a). Similarly, local infusion of a selective competitive NMDA antagonist, CPP (10–204 nmol/rat) into the prefrontal region enhanced the prefrontal dopamine disappearance (Table 3b), whereas a non-NMDA antagonist, GDEE (204 nmol/rat), even at a high dose failed to change dopamine utilization in the prefrontal cortex (Table 3c). None of these agents altered the prefrontal NA disappearance (data not shown).

Effects of combined administration of PCP with MK-801, CPP or Hal on dopamine utilization in the medial prefrontal cortex

PCP (10 mg kg^{-1}, i.p.; Figure 4a and b), MK801 (1.25 mg kg^{-1}, i.p.; Figure 4a) or CPP (204 nmol/rat, intracranially; Figure 5) accelerated the disappearance of dopamine in the medial prefrontal cortex. Both systemic

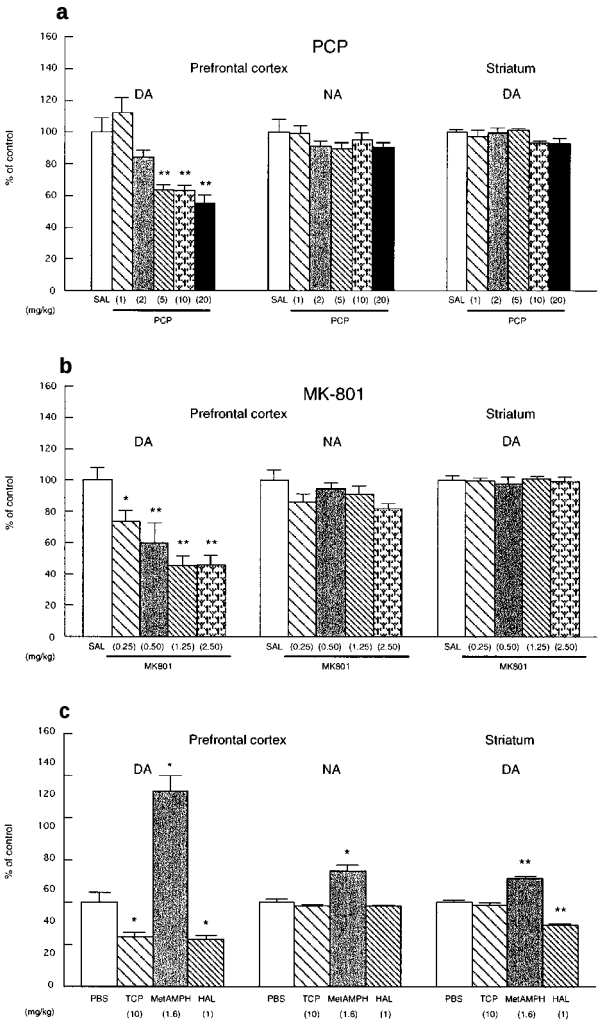

Figure 3 Effects of systemic administration of PCP, TCP, MK-801, MetAMPH and haloperidol (HAL) on dopamine (DA) and NA utilization in the medial prefrontal cortex and striatum of the rat. Animals received PCP (2–20 mg kg^{-1}, i.p.; (a)), MK-801 (0.25–2.5 mg kg^{-1}, i.p.; (b)), TCP (10 mg kg^{-1}, i.p.; (c)), MetAMPH (1.6 mg kg^{-1}, i.p.; (c)), haloperidol (1 mg kg^{-1}, i.p.; (c)) or saline 30 min before being killed. α-MT (250 mg kg^{-1}, i.p.) was injected 35 before decapitation. Results are as presented in Figure 1 ($n=5-7$) and the respective control values were (ng mg^{-1} protein): (a) medial prefrontal cortex, DA 0.49 ± 0.04, NA 2.64 ± 0.21, $n=6$; striatum, DA 89 ± 2, $n=6$; (b), medial prefrontal cortex, DA 0.43 ± 0.04, NA 2.23 ± 0.15, $n=5$; striatum, DA 85 ± 3, $n=7$; (c) medial prefrontal cortex, DA 0.55 ± 0.06, NA 2.67 ± 0.08, $n=6-7$; striatum, DA 82 ± 2, $n=7$. $^{*}P<0.05$, $^{**}P<0.01$ compared to α-MT-treated saline-injected controls. Kruskal-Wallis test followed by the Williams-Wilcoxon test (heterogeneous variance: (c), prefrontal cortex), or ANOVA followed by Duncan's test (homogeneous variance: (a), (b) and (c), striatum) [(a) prefrontal cortex: DA, $F(5, 30)=12.459$, $P<0.01$; NA, $F(5, 30)=0.848$, NS, (b) prefrontal cortex: DA, $F(4, 20)=7.499$, $P<0.01$; NA, $F(5, 20)=2.072$, NS, striatum: DA, $F(4, 20)=0.167$, NS, (c) prefrontal cortex: DA, $\chi^2(3)=21.148$, $P<0.01$; NA, $\chi^2(3)=13.687$, $P<0.01$, striatum: DA, $F(3, 24)=115.698$, $P<0.01$].

(2 mg kg^{-1}, i.p.; Figure 4b) and local (27 nmol/rat; Figure 5) administration of Hal also facilitated the prefrontal dopamine utilization. The doses of the four agents were chosen for this series of interaction experiments because these doses were shown to induce maximal effects on the increase in dopamine utilization (see Figure 3 and Table 3).

The magnitude of the facilitating effects of a combined administration of PCP with either MK-801 (Figure 4a) or CPP (Figure 5) on prefrontal dopamine disappearance was similar

to that found in the animals treated with PCP, MK-801 or CPP alone (Figure 4a and Figure 5). However, when both PCP and Hal were injected into the same animal, cortical dopamine utilization was still accelerated compared to rats receiving PCP or Hal alone (Figure 4b and Figure 5), the two effects being additive.

Table 3 Effects of local infusion of PCP, TCP, CPP or GDEE into the prefrontal cortex on dopamine (DA) utilization in the cortical region of the rat

Infusion (nmol/rat)	*Prefrontal DA* (ng mg^{-1} protein)
a Non-competitive NMDA antagonist	
PBS	0.51 ± 0.02
PCP (10)	0.43 ± 0.03*
PCP (100)	0.30 ± 0.02**
TCP (100)	0.33 ± 0.01**
b Competitive NMDA antagonist	
PBS	0.47 ± 0.03
CPP (10)	0.40 ± 0.05
CPP (70)	0.31 ± 0.02**
CPP (102)	0.32 ± 0.02**
CPP (204)	0.32 ± 0.03**
c Non-competitive, non-NMDA antagonist	
PBS	0.47 ± 0.05
GDEE (204)	0.53 ± 0.04

PCP, TCP, CPP, GDEE or PBS was infused into the medial prefrontal cortex 30 min before cervical dislocation. α-MT (250 mg kg^{-1}, i.p.) was injected 5 min before infusion of various glutamate receptor antagonists or PBS. Results are as presented in Table 1 ($n = 6-8$). $^{*}P<0.05$, $^{**}P<0.01$ compared to α-MT-treated PBS-infused controls. ANOVA followed by Duncan's test or two-tailed Student's t test [(a) PCP, TCP or PBS: prefrontal DA, $F(3, 20) = 20.564$, $P<0.01$, (b) CPP or PBS: prefrontal DA, $F(4, 25) = 4.704$, $P<0.01$, (c) GDEE or PBS: prefrontal DA, $t = 1.034$, NS].

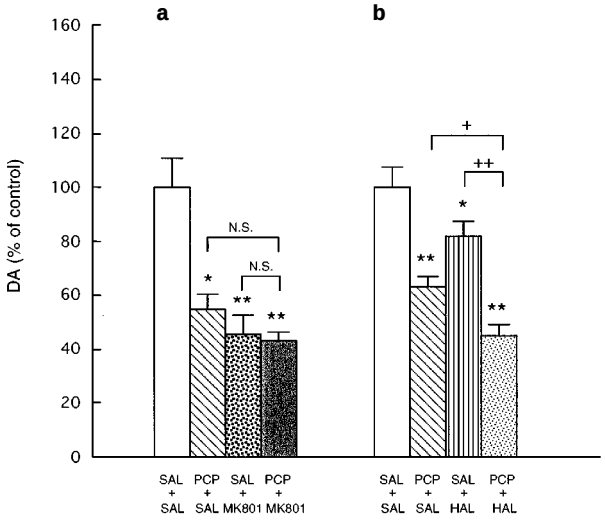

Figure 4 Effects of systemic administration of PCP, MK-801, haloperidol (HAL) or their combination on dopamine (DA) utilization in the medial prefrontal cortex of the rat. Animals received PCP (10 mg kg^{-1}, i.p.; (a) and (b)), MK-801 (1.25 mg kg^{-1} i.p.; (a)), Hal, haloperidol (2 mg kg^{-1}, i.p.; (b)), saline ((a) and (b)) or their combination 30 min before being killed. α-MT (250 mg kg^{-1}, i.p.) was injected 35 min before decapitation. Results are as presented in Figure 1 ($n = 6-9$) and the respective control values were (ng mg^{-1} protein): (a) DA 0.51 ± 0.05, $n = 6$; (b) DA 0.52 ± 0.04, $n = 9$. $^{*}P<0.05$, $^{**}P<0.01$ compared to α-MT-treated saline-injected controls. $^{+}P<0.05$, $^{++}P<0.01$ between the two groups linked with a solid line. ANOVA followed by Duncan's test [(a), $F(3, 20) = 16.106$, $P<0.01$; (b), $F(3, 32) = 17.805$, $P<0.01$].

Effects of intra-prefrontal cortex infusion of NMDA on CPP- and PCP-induced facilitation of dopamine utilization in the medial prefrontal cortex

Intracortical infusion of NMDA (10 or 102 nmol/rat) antagonized the ability of systemic PCP (10 mg kg^{-1}, i.p.) to accelerate the disappearance of dopamine in the medial prefrontal cortex (Figure 6). NMDA by itself produced no significant effects on cortical dopamine utilization (Figure 6).

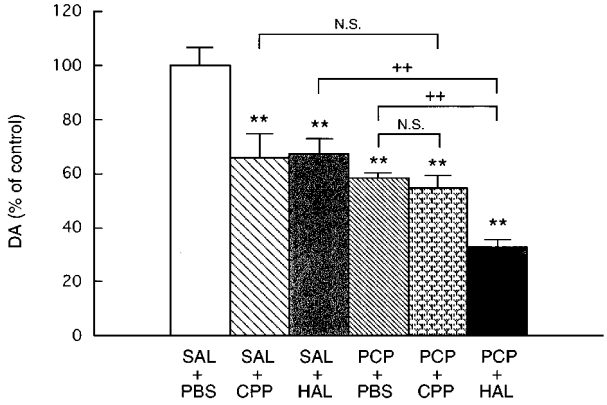

Figure 5 Effects of systemic administration of PCP, intra-prefrontal cortex infusion of CPP or haloperidol (HAL), and their combination on dopamine (DA) utilization in the medial prefrontal cortex of the rat. Animals received PCP (10 mg kg^{-1}, i.p.), CPP (204 nmol/rat) or haloperidol (27 nmol/rat), or their combination 30 min before being killed. α-MT (250 mg kg^{-1}, i.p.) was injected 35 min before decapitation. Results are as presented in Figure 1 ($n = 5$) and the respective control values were (ng mg^{-1} protein): DA 0.52 ± 0.03. $^{*}P<0.05$, $^{**}P<0.01$ compared to α-MT-treated saline-injected PBS-infused controls. $^{+}P<0.05$, $^{++}P<0.01$ between the two groups linked with a solid line. ANOVA followed by Duncan's test [$F(5, 24) = 15.422$, $P<0.01$].

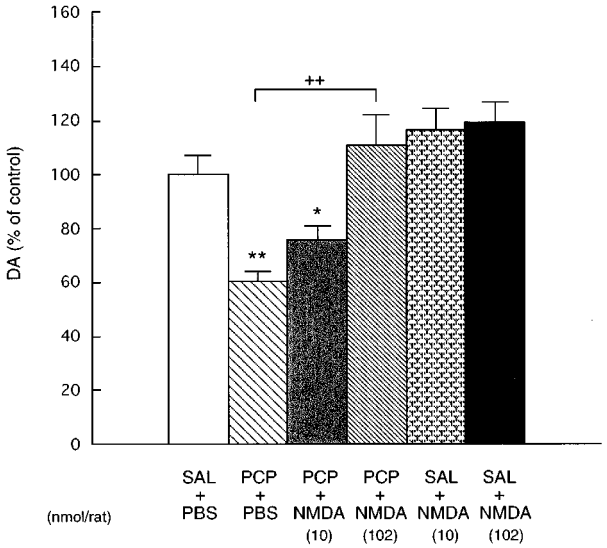

Figure 6 Effects of intra-prefrontal cortex infusion of NMDA on the ability of PCP to facilitate dopamine (DA) utilization in the cortical region of the rat. PCP (10 mg kg^{-1}, i.p.) or saline was administered 30 min, and NMDA (10–102 nmol/rat) or PBS was injected 25 min before death. α-MT (250 mg kg^{-1}, i.p.) was injected 35 min before decapitation. Results are as presented in Figure 1 ($n = 7$) and the respective control values were (ng mg^{-1} protein): DA 0.42 ± 0.03. $^{*}P<0.05$, $^{**}P<0.01$ compared to controls treated with α-MT, PBS and saline. $^{+}P<0.05$, $^{++}P<0.01$ between the two groups linked with a solid line. ANOVA followed by Duncan's test [$F(5, 36) = 10.3957$, $P<0.01$].

Effects of intra-ventricular infusion of D- and L-alanine on PCP-induced facilitation of dopamine utilization in the medial prefrontal cortex

Intraventricular injection of D-alanine (0.2–1.1 μmol/rat) into the medial prefrontal cortex caused a dose-related inhibition of PCP-induced augmentation of dopamine utilization in the cortex (Figure 7a and b) whilst L-alanine (1.1 μmol/rat) was without an effect in this paradigm (Figure 7b). Neither D- nor L-alanine significantly affected cortical dopamine utilization (Figure 7a and b).

Discussion

For the evaluation of dopamine metabolism in this study, we measured dopamine utilization after inhibition of tyrosine hydroxylase together with the levels of dopamine and its metabolites, because estimation of dopamine utilization may reflect electrical activity of dopaminergic neurones which cannot be extrapolated by the tissue or extracellular contents of dopamine or its metabolites alone. For example, dopamine utilization in rat brain tissues has been shown to be accelerated by dopamine receptor antagonists (Scatton, 1977; present study), which acutely augment the firing rates of dopaminergic neurones (Sesack & Bunney, 1987). The tyrosine hydroxylase inhibition method also permits simultaneous monitoring of NA utilization (Sharman, 1981).

In agreement with previous findings (Bowers & Hoffman, 1984; Deutch *et al*., 1987; Rao *et al*., 1990; Nishijima *et al*., 1996), the present data indicate that PCP induces a preferential activation of dopamine metabolism in the prefrontal cortex compared to the striatum. Furthermore, we have demonstrated that the PCP-induced increase in dopamine metabolism is not affected by destruction of ascending noradrenergic neurones and that PCP causes an NMDA- and D-alanine-sensitive facilitation of cortical dopamine utilization. The accelerated disappearance of cortical dopamine evoked by PCP and selective NMDA antagonists were not additive, although a dopamine receptor antagonist, Hal, still accelerated dopamine utilization even in the presence of PCP. The failure of non-NMDA antagonists to enhance cortical dopamine metabolism is consistent with other data (Hala *et al*., 1990; Bubser *et al*., 1995; Jedema & Moghaddam, 1996) and seems to exclude the possibility that the increase in cortical dopamine function produced by PCP and the selected NMDA antagonists is due to a non-specific effect.

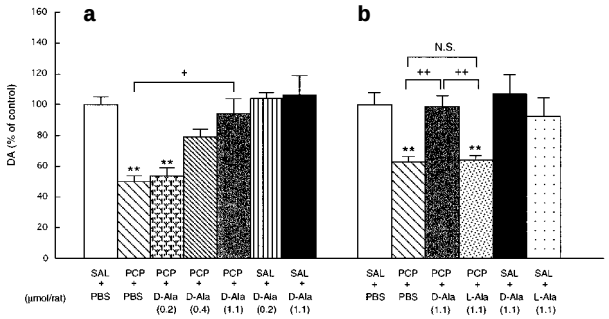

Figure 7 Effects of intra-prefrontal cortex infusion of D- and L-alanine on the ability of PCP to facilitate dopamine (DA) utilization in the cortical region of the rat. PCP (10 mg kg^{-1}, i.p.) or saline was administered 30 min, and D- or L-alanine (0.2–1.1 μmol/rat) or PBS was injected 25 min before death. α-MT (250 mg kg^{-1}, i.p.) was injected 35 before decapitation. Results are as presented in Figure 1 ($n=5-24$) and the respective control values were (ng mg^{-1} protein): (a) DA 0.56±0.03; (b) DA 0.49±0.04. $^{*}P<0.05$, $^{**}P<0.01$ compared to controls treated with α-MT, PBS and saline. $^{+}P<0.05$, $^{++}P<0.01$ between the two groups linked with a solid line. ANOVA followed by Scheffé's test (the different number of animals in each group: (a)) or Duncan's test (the same number of animals in each group: (b)) [(a), $F(6, 73) = 16.873$, $P<0.01$; (b), $F(5, 24) = 6.975$, $P<0.01$].

Evidence has been provided for the regulation of dopamine metabolism by noradrenergic neurones in the cerebral cortex. Thus, a selective NA uptake blocker, desipramine, increases the extracellular release of dopamine in the NA-rich prefrontal cortex but not in the striatum which has only sparse noradrenergic innervation (Carboni *et al*., 1990; Pozzi *et al*., 1994). The effect of desipramine on cortical dopamine was found to be eliminated by lesion of the ascending noradrenergic projections originating from the locus coeruleus (Pozzi *et al*., 1994). Because PCP inhibits NA uptake by rat brain synaptosomes (Garey & Heath, 1976; Smith *et al*., 1977) and elevates cortical levels of a major NA metabolite (Deutch *et al*., 1987), the facilitation of cortical dopamine metabolism by PCP could be mediated indirectly via the modulation of the noradrenergic influence on the dopaminergic neurones in the prefrontal cortex. However, this possibility does not appear to be the major mechanism, because (1) the selective destruction of ascending noradrenergic neurones did not modify the PCP-induced changes in the cortical content of dopamine and its metabolites (Figure 2) and (2) systemic and intra-prefrontal cortex application of PCP accelerated dopamine utilization without affecting cortical NA utilization (Figure 3 and Table 3).

PCP-induced augmentation of dopamine disappearance from the prefrontal cortex is more likely due to NMDA receptor blockade, because both selective non-competitive and competitive NMDA antagonists mimicked the ability of PCP to increase both the levels of dopamine metabolites and the utilization of dopamine in the prefrontal cortex (Tables 1–3 and Figures 3–5). The marked facilitation of dopamine utilization suggests that PCP shares stimulant effects on the firing of mesocortical dopaminergic neurones with selective NMDA antagonists. This idea fits with recent data (Nishijima *et al*., 1994; Hondo *et al*., 1994; Kashiwa *et al*., 1995; Nishijima *et al*., 1996) which showed that NMDA antagonists enhance extracellular release of dopamine in the medial prefrontal cortex in a tetrodotoxin-reversible manner.

A pivotal role of the NMDA receptor in the PCP-induction of hyperdopaminergic activity in the prefrontal cortex is further corroborated by the present findings that local infusion of NMDA or an allosteric NMDA agonist, D-alanine, but not its L-isomer, attenuated the ability of PCP to enhance prefrontal dopamine utilization (Figures 6 and 7). These data seem to be in line with the behavioural observations that stimulation of the NMDA receptor function by its allosteric agonists, D-serine and D-alanine, reduce abnormal behaviour elicted by PCP (Contreras, 1990; Tanii *et al*., 1991; 1994) or MK-801 (Contreras, 1990).

Because both systemic administration and intra-prefrontal cortex infusion of PCP caused a similar increase in prefrontal dopamine utilization, PCP may modulate dopaminergic neuronal activity by blockade of the NMDA receptor at the level of the nerve terminal areas of mesocortical dopaminergic neurones. In accordance with this possibility, the acceleration of the disappearance of dopamine observed in the prefrontal cortex after i.p. injection of PCP was antagonized by local injection of NMDA into the cortical region (Figure 6). Furthermore, local infusion of selective competitive or non-competitive antagonists of the NMDA receptor into the

prefrontal cortex, but not into the VTA (the source of the cortical dopaminergic afferents), increased cortical dopamine metabolism (Tables 2 and 3).

In contrast to the prefrontal cortex, systemic injection of PCP reduced tissue contents of DOPAC without significantly altering dopamine levels in the striatum 60 min thereafter (Figure 1), whereas selective non-competitive NMDA antagonists, MK-801 and TCP, produced no change in DOPAC (Table 1). This discrepancy suggests that the PCP-induced alterations in striatal dopamine metabolism is not caused solely by the blockade of the NMDA receptor. PCP, but not MK-801 or TCP, has been shown to have high affinity for the dopamine transporter system *in vivo* (Maurice *et al*., 1991) and to be approximately equipotent with amphetamine as a dopamine uptake inhibitor in striatal tissue *in vitro* (Bowyer *et al*., 1984). However, effectiveness of PCP as a dopamine releaser is about one-tenth of the potency of amphetamine (Bagchi, 1981; Vickroy & Johnson, 1982; Bowyer *et al*., 1984). These observations, together with the general agreement that the tissue content of DOPAC is primarily derived from dopamine by the effect of monoamine oxidase within the presynaptic site (Zetterström *et al*., 1988), indicate that the decrease of striatal dopamine metabolites after administration of a high dose of PCP might, at least in part, be associated with inhibition of dopamine uptake by this drug. This hypothesis agrees with the results of *in vivo* dialysis experiments indicating that systemic PCP induces a diminution of extracellular DOPAC release, with a slight increase in dopamine contents in the dialysate in the striatum, while systemic MK-801 leads to a slight elevation of both extracellular dopamine and DOPAC levels (Nishijima *et al*., 1994; 1996; Kashiwa *et al*., 1995). The lack of effect of PCP, MK-801 and TCP on striatal dopamine utilization also represents a striking contrast to their apparent facilitation of the index of dopamine metabolism in the prefrontal cortex, suggesting that these drugs do not elicit marked changes in impulse flow in the striatal dopaminergic neurones.

Conclusions

The present findings demonstrate that, like selective NMDA antagonists, PCP causes a preferential facilitation of dopamine metabolism in the prefrontal cortex. The antagonism by NMDA receptor stimulation of PCP-induced cortical hyperdopaminergic activity (present study) as well as neuroleptic-resistant abnormal behaviour (Contreas, 1990; Tanii *et al*., 1991; 1994) further supports the idea that the co-agonist site of the NMDA receptor could be a suitable target for the development of novel antipsychotic agents (Deutsch *et al*., 1989; Nishikawa *et al*., 1991).

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