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Interactions between cocaine and (−)-DS 121: studies with 2-deoxyglucose autoradiography and microdialysis in the rat brain

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Abstract

(−)-DS 121 [*S*-(−)-3-(3-cyanophenyl)-*N*-*n*-propyl piperidine], a dopamine autoreceptor preferring antagonist, has been shown to stimulate locomotor activity and induce conditioned place preference. However, the drug fails to facilitate intracranial self-stimulation or substitute for cocaine in cueing experiments, and it blocks cocaine self-administration. In the present study using 2-deoxyglucose autoradiography, (−)-DS 121 (at 50 but not 15 mg/kg i.p.) significantly and selectively increased local cerebral glucose utilization in the olfactory cortex, medial and lateral septum, hippocampal areas, substantia nigra pars reticulata, caudate, and mammillary body. Local cerebral glucose utilization was depressed in caudal areas of the cortex. Interestingly, however, both doses of (−)-DS 121 blocked the increases in local cerebral glucose utilization produced by 5 mg/kg i.v. cocaine. The present study also evaluated the effects of (−)-DS 121 on extracellular striatal dopamine levels using microdialysis in freely moving rats. By itself, 15 mg/kg of (−)-DS 121 increased extracellular striatal dopamine levels to approximately 300% of controls. Cocaine (5 mg/kg i.v.) produced a 370% increase in striatal dopamine levels. When rats were pretreated with (−)-DS 121, a subsequent dose of cocaine augmented the increase in extracellular striatal dopamine to 870% of controls. The results support the contention that (−)-DS 121 possesses weak cocaine-mimetic effects and that its antagonism of cocaine's subjective effects are due to interactions with dopamine at postsynaptic sites. It is hypothesized that, like other preferential autoreceptor antagonists, (−)-DS 121 may be useful as a pharmacotherapy in drug addiction.

Keywords: 2-Deoxyglucose autoradiography; Microdialysis; Dopamine; Autoreceptor antagonist; Cocaine; Drug addiction

1. Introduction

Cocaine addiction is a widespread problem in today's society, and the strong addictive properties of the drug make cocaine abuse a habit hard to discontinue. The highly rewarding properties of cocaine cause substantial psychological withdrawal symptoms, and thus far no therapeutic alleviation of these symptoms is available (Kleber, 1995; Roberts and Ranaldi, 1995).

Pharmacologically, cocaine binds to a specific site at the dopamine transporter, rendering it unable to transport dopamine back into the nerve terminal (Ritz et al., 1987; Uhl, 1992). Consequently, dopamine neuronal transmission is enhanced. The mesocorticolimbic dopamine system is of particular importance in mediating cocaine's effects (Kuhar et al., 1991), especially the drug's rewarding properties, as shown in lesion and cocaine self-administration experiments in rats (Evenden and Ryan, 1988; Roberts and Koob, 1979).

The recent cloning of the dopamine D_3 receptor by Sokoloff et al. (1990) has opened new insights into the dopamine neuronal system and possible research strategies for the pharmacotherapy of drug addiction. Dopamine D_3 selective/preferring agonists, for example, 7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT), have been shown to reduce cocaine self-administration in rats (Caine and Koob, 1993). The doses of the agonist required to show this effect were, by themselves, not reinforcing. Problems with applying this dopamine D_3 approach are (a) the low selectivity of 7-OH-DPAT and (b) more importantly, that dopamine D_2 autoreceptor stimulation results in inhibition of locomotor activity due to decreased dopamine neurotransmission (Caine and Koob, 1993; Svensson et al., 1994). Moreover, the use of a reinforcement schedule where every lever press is rewarded, as in the Caine and Koob (1993) study, could lead to a decrease

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Table 1
Corresponding LCGU values to Fig. 1, values are expressed in μmol/100 g/min. Brain area abbreviations are according to the brain atlas by Paxinos and Watson (1986). Significances are noted by symbols, where [a] $P < 0.05$ and [b] $P < 0.01$.

Name	Saline	Cocaine	DS121 (15 mg)		DS121 (50 mg)	
	control	control	Control	+ Cocaine	Control	+ Cocaine
Cerebral cortex						
Drsl Pfcx	62.5 ± 2.8	76.4 ± 6.3	62.1 ± 3.2	69.2 ± 4.4	81.4 ± 11.8	77.0 ± 7.9
Mdl Pfcx	64.8 ± 3.4	75.6 ± 5.9	66.7 ± 4.4	67.7 ± 4.0	75.6 ± 4.6	67.3 ± 6.0
Ventrl Pfcx	74.8 ± 3.0	90.2 ± 5.4	74.5 ± 5.3	78.1 ± 4.2	86.3 ± 4.9	78.2 ± 6.0
Olf Cx	69.6 ± 3.0	73.0 ± 6.4	71.5 ± 5.5	62.0 ± 4.7	88.5 ± 9.3 [a]	71.2 ± 4.6
Frntl Cx	70.9 ± 3.3	82.6 ± 5.2 [b]	71.1 ± 2.7	69.8 ± 5.2	73.3 ± 6.2	61.3 ± 4.7
Ant Cing	76.1 ± 3.4	81.7 ± 5.1	74.6 ± 2.2	71.3 ± 5.7	87.6 ± 6.9	72.5 ± 4.8
Mo Cx	72.2 ± 2.8	87.3 ± 5.9 [a]	72.5 ± 2.2	74.6 ± 5.6	76.8 ± 6.6	64.4 ± 5.1
SNS Cx	73.7 ± 2.9	81.4 ± 4.9	71.4 ± 2.3	71.1 ± 6.0	74.1 ± 6.9	61.9 ± 4.8
Pyr Cx	84.5 ± 3.9	82.9 ± 6.1	80.1 ± 1.8	73.8 ± 6.0	87.1 ± 6.0	79.7 ± 6.0
Par Cx	70.1 ± 3.5	79.3 ± 4.4	68.6 ± 2.7	68.8 ± 5.4	73.5 ± 5.8	63.0 ± 5.2
Aud Cx	84.8 ± 2.1	92.4 ± 6.2	81.2 ± 3.4	80.1 ± 6.4	73.9 ± 5.9	70.3 ± 4.2 [a]
Temp Cx	84.6 ± 2.6	75.1 ± 4.2	78.3 ± 3.0	68.3 ± 5.3 [a]	73.4 ± 5.1 [b]	70.3 ± 3.5 [a]
Rsplen Cx	74.5 ± 2.6	72.7 ± 5.6	68.7 ± 3.6	60.3 ± 3.7 [a]	59.4 ± 4.7 [a]	56.2 ± 4.0 [a]
Str XVII	84.3 ± 3.8	72.0 ± 4.6 [a]	75.0 ± 4.4	64.8 ± 3.9	69.1 ± 5.1 [a]	62.0 ± 2.9 [a]
Str XVIII	85.8 ± 3.7	75.1 ± 4.8 [b]	76.9 ± 5.2	67.3 ± 3.4 [a]	71.7 ± 3.9 [a]	64.2 ± 3.0 [a]
Ent Cx	57.5 ± 3.6	52.4 ± 4.0	56.8 ± 4.1	54.5 ± 3.3	66.6 ± 6.5	60.3 ± 4.4
Limbic system						
Ant Accmbn	64.4 ± 2.8	69.5 ± 4.8	72.9 ± 2.7	64.4 ± 5.9	73.6 ± 8.0	65.2 ± 5.2
N Accmbn	51.3 ± 2.7	54.7 ± 5.2	52.3 ± 2.6	46.0 ± 6.3	57.4 ± 5.4	51.5 ± 5.9
Olf Tub	75.5 ± 2.5	75.3 ± 6.2	72.9 ± 1.6	68.1 ± 5.5	75.3 ± 5.1	70.8 ± 5.8
Fornix	30.9 ± 3.7	35.0 ± 2.3	39.3 ± 2.8	33.9 ± 5.0	41.9 ± 3.9	36.2 ± 3.6
Cn Amyg	66.4 ± 4.0	71.2 ± 7.0	64.1 ± 4.0	56.7 ± 5.2	62.5 ± 4.7	60.1 ± 5.7
Hippo I	48.1 ± 3.5	46.8 ± 4.3	39.3 ± 2.5	43.0 ± 5.5	58.1 ± 7.0	58.5 ± 6.4
Hippo III	48.2 ± 2.8	46.8 ± 3.7	41.6 ± 2.7	42.7 ± 4.7	65.6 ± 9.2 [a]	66.1 ± 7.8 [a]
Hippo IV	50.7 ± 3.1	49.3 ± 3.4	45.3 ± 3.7	45.6 ± 6.0	69.0 ± 6.1 [a]	67.1 ± 7.3 [a]
Dentate	53.2 ± 3.4	55.0 ± 4.7	47.2 ± 3.5	50.9 ± 5.2	66.0 ± 5.8 [b]	66.8 ± 7.0 [b]
Dent Mol	58.5 ± 3.4	61.0 ± 4.7	52.1 ± 3.2	55.2 ± 5.4	71.1 ± 6.3	72.8 ± 7.4 [b]
Hippo Dnd	56.9 ± 3.7	58.7 ± 4.9	50.9 ± 3.6	53.1 ± 5.6	68.4 ± 7.9	70.2 ± 6.3
Bla Amyg	62.1 ± 2.3	59.5 ± 5.7	56.5 ± 3.4	51.7 ± 5.5	58.1 ± 5.1	57.4 ± 6.3
Me Amyg	44.4 ± 2.2	42.3 ± 3.7	41.4 ± 2.5	36.2 ± 5.0	49.6 ± 5.7	48.9 ± 4.5
Mamm Body	75.1 ± 3.5	89.0 ± 4.9 [b]	85.0 ± 2.7	68.6 ± 8.1	90.3 ± 7.2 [b]	82.3 ± 3.9
Thalamus						
Av Thal	65.5 ± 2.7	75.4 ± 5.5	63.1 ± 3.0	62.0 ± 3.4	57.2 ± 6.0	54.4 ± 4.4 [b]
VI Thal	67.6 ± 3.0	80.2 ± 4.9	72.4 ± 3.6	70.8 ± 3.1	73.3 ± 6.7	64.2 ± 5.9
L Habenula	75.1 ± 3.2	68.6 ± 3.9	79.3 ± 1.8	68.1 ± 5.8	84.6 ± 7.0	66.8 ± 5.6
M Habenula	45.5 ± 3.6	46.9 ± 3.2	48.9 ± 3.8	44.6 ± 5.3	50.6 ± 4.7	50.3 ± 6.1
Lateral Gen	56.2 ± 2.3	59.3 ± 3.8	54.5 ± 4.4	53.9 ± 4.3	57.0 ± 4.7	51.7 ± 3.9
Medial Gen	66.1 ± 1.8	73.1 ± 4.1	66.4 ± 4.9	63.5 ± 6.0	67.8 ± 6.3	60.2 ± 4.4
Monoaminergic nuclei						
VTA	53.5 ± 1.1	55.3 ± 2.7	58.3 ± 3.0	50.8 ± 4.2	60.0 ± 6.3	50.6 ± 4.5
SNPR	46.6 ± 1.5	58.6 ± 2.8 [a]	50.1 ± 3.6	51.0 ± 4.0	70.2 ± 6.8 [a]	68.0 ± 4.3 [a]
SNPC	57.0 ± 1.7	63.4 ± 3.9	60.3 ± 4.7	58.0 ± 4.5	64.8 ± 5.5	59.3 ± 2.7
D Raphe	51.2 ± 1.0	56.9 ± 4.3	51.5 ± 3.4	53.1 ± 2.7	54.2 ± 5.2	54.7 ± 3.3
M Raphe	57.3 ± 2.2	49.1 ± 4.3	51.9 ± 6.1	46.5 ± 2.6	51.9 ± 6.9	38.3 ± 2.6 [a]
Locus	36.3 ± 1.6	38.7 ± 2.7	37.4 ± 3.5	33.3 ± 4.5	32.9 ± 4.0	32.7 ± 3.0
Medial basal cholinergic nuclei						
M						
Septum	51.7 ± 2.4	52.6 ± 3.5	54.9 ± 2.2	53.0 ± 4.9	72.3 ± 7.2 [a]	62.8 ± 4.3 [b]
DBB	55.6 ± 2.8	59.3 ± 3.7	61.0 ± 1.8	55.0 ± 4.8	69.8 ± 6.9	62.9 ± 4.0
Basalis	46.3 ± 3.3	51.8 ± 3.9	49.6 ± 3.1	47.6 ± 6.7	50.8 ± 4.7	46.8 ± 5.1
Extrapyramidal regions						
Caudate	73.1 ± 2.8	91.3 ± 5.4 [a]	81.8 ± 2.2	81.3 ± 6.9	93.8 ± 7.9 [a]	79.1 ± 5.9
Globus	44.3 ± 4.0	61.9 ± 5.6	50.6 ± 3.6	51.7 ± 6.6	57.7 ± 5.7	49.8 ± 5.8
Sub Thal	60.1 ± 3.6	69.2 ± 6.8	60.7 ± 4.7	62.0 ± 3.6	63.7 ± 4.0	63.8 ± 4.1

Table 1 (continued)

Name	Saline control	Cocaine control	DS121 (15 mg)		DS121 (50 mg)	
			Control	+Cocaine	Control	+Cocaine
Other diencephalic structures						
GCC	26.1 ± 1.9	29.9 ± 1.5	25.5 ± 1.9	23.2 ± 3.6	30.2 ± 4.9	22.5 ± 1.9
L Septum	40.6 ± 2.0	45.8 ± 3.2	47.3 ± 1.5	49.8 ± 5.1	60.5 ± 7.1 [a]	53.5 ± 4.2 [a]
L Hyp Thal	45.8 ± 2.2	43.7 ± 3.3	44.6 ± 1.7	40.7 ± 4.7	50.9 ± 4.6	45.8 ± 3.8
M Hyp Thal	41.5 ± 2.5	41.7 ± 3.6	40.4 ± 1.9	37.1 ± 5.2	46.0 ± 5.3	44.8 ± 4.3
Sc Out L	63.1 ± 3.6	57.9 ± 4.8	55.4 ± 4.4	52.9 ± 5.5	53.1 ± 5.8	48.0 ± 3.9
Sc Int L	60.3 ± 1.3	59.2 ± 4.2	57.1 ± 3.7	50.8 ± 5.5	52.5 ± 4.9	48.3 ± 3.7
IC	79.1 ± 6.1	78.3 ± 4.4	74.6 ± 6.5	69.0 ± 5.9	85.1 ± 8.4	69.5 ± 2.8
Brainstem						
IP	66.4 ± 4.8	61.4 ± 5.3	72.8 ± 6.9	63.6 ± 4.1	79.0 ± 6.7	69.7 ± 2.3
Pag	40.5 ± 0.7	45.1 ± 2.7	43.2 ± 2.7	39.3 ± 3.8	40.5 ± 4.1	39.8 ± 2.8
D Tgmntl	60.7 ± 1.7	62.6 ± 3.2	57.0 ± 5.2	54.8 ± 3.4	63.5 ± 6.6	56.3 ± 4.1
Olive	77.5 ± 3.3	71.1 ± 2.3	74.4 ± 4.9	66.7 ± 4.6 [b]	80.8 ± 5.9	66.3 ± 2.4 [b]
Vestib N	80.8 ± 4.2	72.9 ± 2.7	69.7 ± 7.9	71.5 ± 5.3	84.8 ± 4.9	81.2 ± 4.3
Crblm	46.3 ± 2.0	58.1 ± 2.2	47.3 ± 2.9	51.3 ± 4.0	51.9 ± 4.3	50.7 ± 4.2
Spinal cord						
D Horn	47.2 ± 3.3	52.7 ± 2.7	46.3 ± 2.7	47.8 ± 3.2	48.2 ± 4.9	47.1 ± 4.3
V Horn	38.6 ± 3.2	41.8 ± 3.1	41.5 ± 3.3	40.4 ± 4.0	42.8 ± 4.1	39.4 ± 3.0

[a] $P < 0.05$. [b] $P < 0.10$.

in response rate resulting from possible rewarding effects of 7-OH-DPAT.

Recently, the pharmacology of the dopamine autoreceptor preferring antagonist (−)-DS 121 was described (Sonesson et al., 1993, 1994, Waters et al., 1994; Clark et al., 1995). In vitro binding studies showed that (−)-DS 121 has about 4 times higher affinity for the dopamine D_3 receptor compared to the dopamine D_2 receptor, and shows less than 50% inhibition at 1 μM for D1, D4, α_1, α_2, β, 5-HT, 5-HT_{1D}, 5-HT_2, muscarinic, acetylcholine, benzodiazepine and opiate receptors (Sonesson et al., 1994).

Behavioral experiments have demonstrated that (−)-DS 121 acts as a weak stimulant by preferentially blocking dopamine autoreceptors, resulting in increased dopamine release (Sonesson et al., 1994). The importance of (−)-DS 121's slight dopamine D_3 preference for its behavioral profile is unknown; however, it has been shown that dopamine D_3 selective antagonists (such as U-99194A) produce behavioral stimulation (Waters et al., 1993b). Unlike classical dopamine antagonists, (−)-DS 121 increases locomotor activity and induces conditioned place preference, but does not facilitate intracranial self-stimulation, indicating low or no addiction liability (Kling-Petersen et al., 1995a,b). Smith et al. (1996) showed that (−)-DS 121 is effective in decreasing cocaine self-administration in rats, but does not substitute for cocaine in rats trained to lever-press for cocaine. Also, Clark et al. (1995) showed that (−)-DS 121 failed to substitute for cocaine or *d*-amphetamine in the cueing paradigm. While stimulating locomotor activity in habituated rats, (−)-DS 121 blocked the hyperactivity induced by either *d*-amphetamine (Svensson et al., 1993) or the dopamine receptor agonist DP-5,6-ADTN (Waters et al., 1994).

This distinct profile of (−)-DS 121 (dopamine D_3 vs. D_2 preference, mild stimulant activity, autoreceptor preference) make this drug an interesting candidate in evaluating a possible strategy for the pharmacological treatment of addictive drug abuse. Indeed, other preferential autoreceptor antagonists have been demonstrated to have potential utility in treatment of drug abuse (Piercey et al., 1992; Callahan et al., 1992; Richardson et al., 1993; Casey et al., 1996). Microdialysis in vivo demonstrates that (−)-DS 121 dramatically increases dopamine release (Sonesson et al., 1994). This effect is not likely due to dopamine D_3 receptor binding properties, but rather to the D_2 autoreceptor antagonism properties similar to those for (+)-AJ 76 and (+)-UH 232 (Svensson et al., 1986). This hypothesis is based on the fact that the selective dopamine D_3 receptor antagonist U-99194A produces hyperactivity over a wide dose range without affecting dopamine release (Waters et al., 1993b).

To investigate the interaction between (−)-DS 121 and cocaine on a neurochemical level, 2-deoxyglucose autoradiography and microdialysis studies were performed in male rats. The autoradiography revealed that (−)-DS 121 is able to block the effects of cocaine on local cerebral glucose utilization, which correlates with functional brain activity (Sokoloff, 1977). To further characterize (−)-DS 121's potential use in the treatment of drug addiction, we followed the time course of the 2-deoxyglucose treatment paradigm to investigate the interaction of cocaine and (−)-DS 121 on dopamine release in the striatum, a major

dopamine projection field, using microdialysis in freely moving rats.

2. Materials and methods

2.1. 2-Deoxyglucose autoradiography

The 2-deoxyglucose autoradiography used was modified from the method described by Sokoloff (1977). Charles River (Portage, MI, USA) Sprague-Dawley male rats (250–350 g) were anesthetized with a fluothane/NO_2 mix and fitted with arterial, venous, and intraperitoneal cannulae. Following cannulation, the animals were lightly restrained in a Brain Tree experimental containment unit and allowed to recover for a minimum of 30 min. During the experiment, rats were kept in the restrainer and not further anesthetized. After the recovery period, the animals were injected with either drug or saline. (−)-DS 121 (15 mg/kg or 50 mg/kg) or saline (0.9%) was injected i.p. 15 min prior to 2-deoxyglucose administration. Cocaine (5 mg/kg) or saline was injected i.v. 2 min prior to an i.v. injection of 25 μCi of [^{14}C]2-deoxyglucose (New England Nuclear, Boston, MA, USA). Arterial blood samples were collected over a 45-min time period, after which the rats were killed. The blood plasma was analyzed for radioactivity and glucose concentration and entered into the Sokoloff equation. Brains were rapidly removed, frozen over liquid nitrogen, and sectioned on a Leitz 1720 cryostat. The 20 μm sections were positioned on 18 mm^2 cover glass, mounted on cardboard using double-sided tape, and exposed to Kodak SB-5 X-ray film with [^{14}C] standards (Amersham, Arlington Heights, IL, USA) for 6 days. The resulting autoradiograms were developed and analyzed for local cerebral glucose utilization using an Amersham RAS 1000 image analysis system. Brain areas were identified according to the brain atlas of Paxinos and Watson (1986). Quantitative values of local cerebral glucose utilization were expressed in μmol/100 g per min. Average glucose utilizations within each treatment group were statistically compared with a SAS statistical program (ANOVA and protected *t*-tests).

2.2. Microdialysis

Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) were housed in groups of 3 in a light/dark and temperature-controlled environment. Food and water were available ad libitum. Animals were anesthetized with Chloropent (3 mg/kg i.p.) and mounted into a stereotaxic frame. Four anchoring screws were placed into the scull and a burr hole drilled for implantation of a concentric dialysis probe (Benloucif and Galloway, 1991) into the anterior striatum (coordinates: L = +3 mm, A = +0.7 mm, V = −7 mm, according to the brain atlas by Paxinos and Watson (1986)). Length of exposed membrane (Hospal, France) was 4 mm. The probe was secured with dental cement (Hygenic, Cleveland, OH, USA) and the animals were allowed to recover for 36 h prior to the experiment. Probes were prefilled with artificial cerebral spinal fluid, containing (in mM): 145 Na^+, 2.7 K^+, 1.0 Mg^+, 1.2 $Ca2^+$, pH 7.3, maintained with 2 mM phosphate buffer. The inlet and outlet lines were sealed during the recovery period.

On the day of the experiment, the probe inlet line was connected to a CMA infusion pump and the outlet line to a BAS fraction collector. Artificial cerebral spinal fluid was constantly perfused at a flow rate of 2 μl/min. Dialysates were collected every 20 min and 20 μl injected into the chromatography system for analysis of extracellular dopamine levels. The analysis was performed by high performance liquid chromatography with electrochemical detection (HPLC-EC), essentially as described by Waters et al. (1993a). After a stable dopamine baseline was established, animals were either injected with cocaine, (−)-DS 121, or (−)-DS 121 followed by cocaine. Both drugs were dissolved in 0.9% saline. Drug doses and routes of administration were 5 mg/kg i.v. for cocaine and 15 mg/kg i.p. for (−)-DS 121.

At the end of each experiment, the brain was rapidly removed and stored at −80°C. The brain was later sectioned into 20 μm sections on a Leitz cryostat for verification of the probe placement. Data were expressed as percentage increase over control (baseline). Statistical significance of the data was assessed by using one-way ANOVA and Fisher's Protected Least Significant Difference. (−)-DS 121 was synthesized by Sonesson and coworkers, University of Goteborg, Sweden. Cocaine was obtained from Sigma (St. Louis, MO, USA).

3. Results

3.1. 2-Deoxyglucose autoradiography

Cocaine (5 mg/kg i.v.) caused stimulation in glucose metabolism in the frontal and motor cortices, substantia nigra pars reticulata, caudate, and mammillary body, similar to results reported by Porrino et al. (1988). Depression in glucose metabolism selectively occurred in the striate cortices 17 and 18 (Table 1).

The lower dose of (−)-DS 121 (15 mg/kg i.p.) did not significantly alter metabolism in any brain region examined (Table 1, Fig. 1). The higher dose, however, showed mixed effects (Table 1, Fig. 1). (−)-DS 121 (50 mg/kg i.p.) increased local cerebral glucose utilization in the olfactory cortex, medial and lateral septum, CAIII, CAIV, dentate areas of the hippocampus and, like cocaine, in the substantia nigra pars reticulata, caudate, and mammillary body. This dose of (−)-DS 121 also caused depression of local cerebral glucose utilization in caudal areas of the cortex, including the temporal, retrosplenial cortices.

The stimulant effects of cocaine were not evident in the combination treatment, suggesting a blockade of cocaine's effect by (−)-DS 121. With the lower dose of (−)-DS 121, cocaine effects were completely antagonized. However, depressions were found in the temporal, retrosplenial, and striate cortices, as was observed with 50 mg of (−)-DS 121 alone, and additionally in the olivary nucleus. In the combination of the higher dose of (−)-DS 121 and cocaine, the effects were mixed. All of cocaine's stimulant effects were antagonized. Local cerebral glucose utilization was increased in the substantia nigra pars reticulata, medial and lateral septum, and hippocampal areas (CAIII, CAIV, and dentate), similar to the increases seen with (−)-DS 121 alone. Depression in local cerebral glucose utilization was evident in the AV thalamus, medial raphe and olive. The auditory, temporal, retrosplenial and striate cortices were also depressed as seen with (−)-DS 121 (50 mg/kg) alone.

3.2. Microdialysis

Cocaine (5 mg/kg i.v.) significantly increased extracellular dopamine levels to $366 \pm 25\%$ ($P = 0.027$) of baseline controls (Fig. 2 and Fig. 3). Gross behavioral observations also revealed an immediate increase in motor activity with stereotyped behavior. The duration of the cocaine effect lasted about 20 min, then dopamine values returned to baseline levels (Fig. 2).

Similar to cocaine, (−)-DS 121 (15 mg/kg i.p.) produced significant increases in dopamine levels to maximally $297 \pm 29\%$ ($P = 0.012$, Fig. 3) of baseline. Rats injected with (−)-DS 121 also showed increases in motor activity with sniffing and head weaving. The increases in extracellular striatal dopamine produced by (−)-DS 121 tended to be longer in duration than those produced by cocaine. Additionally, the magnitude of the effect of (−)-DS 121 seemed to be consistently lower than the effect of

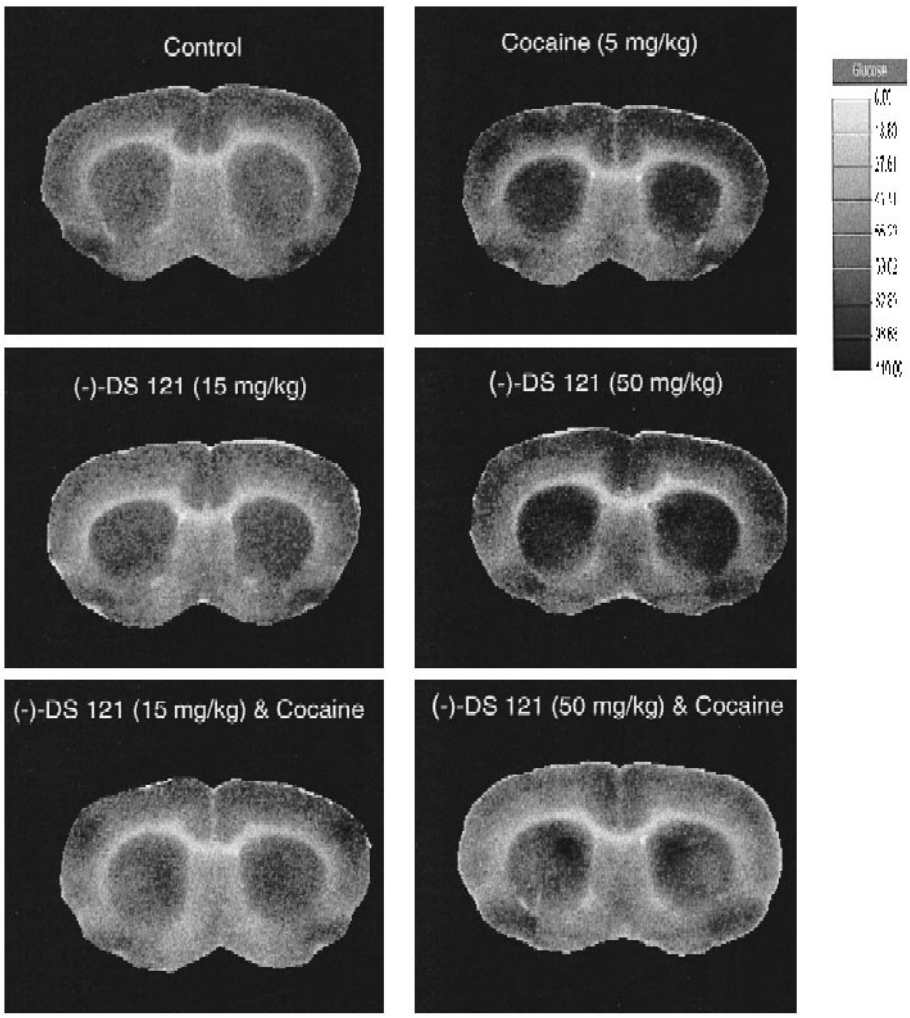

Fig. 1. Autoradiograms showing the effects of (−)-DS 121 (15 or 50 mg/kg, i.p.) and cocaine (5 mg/kg, i.v.) on local cerebral glucose utilization (LCGU) in the rat brain. Representative pictures show glucose changes at the caudate level. Administration of either (−)-DS 121 or cocaine stimulates LCGU in the caudate and cortical areas. When both drugs are given in combination, the increases observed with either drug alone are no longer evident.

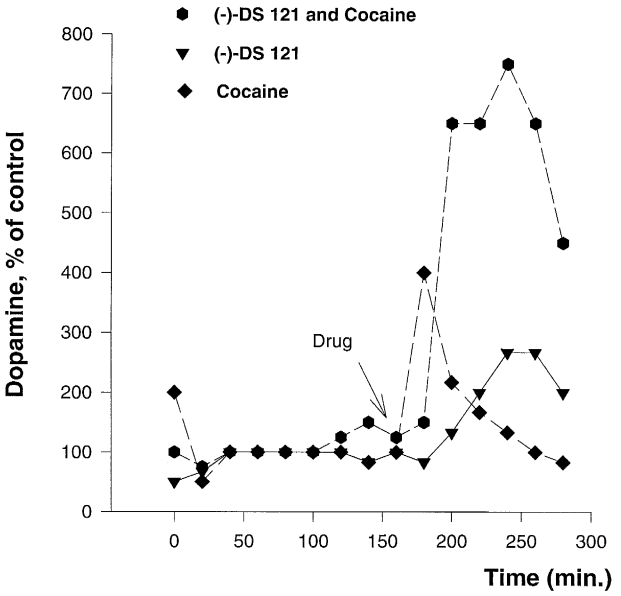

Fig. 2. Typical time course of a dialysis experiment. One to two hours following perfusion of the probe, DA levels stabilize. The graphs show the relative duration of the drug effect on extracellular DA. Cocaine has a short time of action, DA levels quickly return to baseline values. (−)-DS 121's effect alone was similar, however, the combination of (−)-DS 121 and cocaine resulted in a prolonged increase in extracellular DA. Cocaine was injected at 160 min, while (−)-DS 121 was injected 13 min before.

cocaine on extracellular dopamine, but this tendency did not reach statistical significance.

When rats were pretreated with (−)-DS 121 (15 mg/kg i.p.) 13 min prior to cocaine injection, extracellular levels of dopamine increased to 924 ± 120% ($P < 0.0001$) of

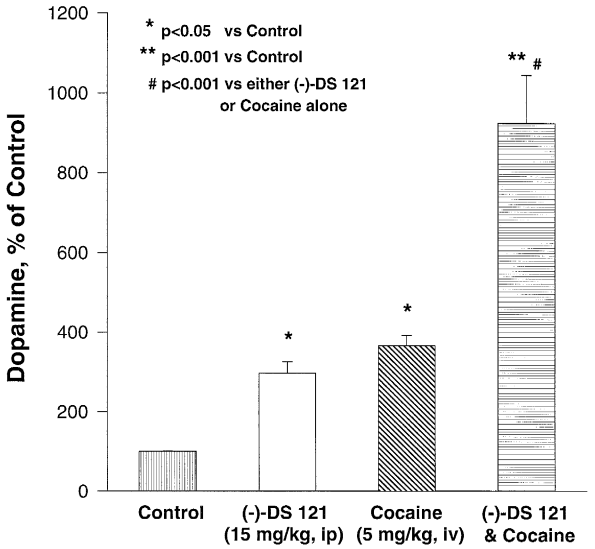

Fig. 3. Administration of (−)-DS 121 (15 mg/kg, i.p.) and cocaine (5 mg/kg, i.v.) significantly increased extracellular striatal levels of dopamine (DA) to 297 ± 29% and 366 ± 25% ($n = 4–5$, $^{*}P < 0.05$), respectively. When given in combination, these doses of (−)-DS 121 and cocaine significantly increased striatal DA to 924 ± 120% ($n = 6$, $^{**}P < 0.001$), significantly higher than either drug alone. Data represent means ± S.E.M. for the fraction collected 40–60 min post-injection.

controls. This effect was significantly higher than the increase produced by either treatment alone ($P < 0.0001$ vs. (−)-DS 121 or cocaine alone).

4. Discussion

This study investigated the neurochemical interaction between (−)-DS 121 and cocaine using microdialysis and [^{14}C]2-deoxyglucose autoradiography in the rat.

The increases in local cerebral glucose utilization we observed in this study with cocaine injected 2 min prior to 2-deoxyglucose were slightly stronger, but similar, in distribution as previously observed when cocaine was injected 5 min prior to [^{14}C]2-deoxyglucose (Casey et al., 1996). The differences are likely due to the short duration of action of cocaine (Pan et al., 1991); thus, the present 2-min preinjection protocol would allow more time for cocaine's peak concentration to promote 2-deoxyglucose accumulation into stimulated neurons than would the 5-min preinjection protocol in the Casey et al. (1996) study.

There was no stimulation of local cerebral glucose utilization in the nucleus accumbens by cocaine, even in its more anterior segments. This result agrees with Casey et al. (1996), but differs from the experiments of Porrino et al. (1988). Since our current protocol used a very short preinjection period similar to that of Porrino et al. (1988), pharmacokinetic considerations cannot be used to explain the differences between results in nucleus accumbens in different laboratories. However, it is still possible that the nucleus accumbens local cerebral glucose utilization increases observed by Porrino et al. (1988) occurred in areas still more anterior than the level we refer to as anterior accumbens (1.6 mm anterior to bregma, plate 12 of Paxinos and Watson, 1986).

The phenylpiperidine (−)-DS 121 is a new dopamine autoreceptor preferring antagonist with a slight preference for the dopamine D_3 receptor vs. the dopamine D_2 receptor. (−)-DS 121 increased local cerebral glucose utilization in several brain regions, similar to the action of cocaine. In this sense, (−)-DS 121 is a stronger stimulant than the 2-aminotetralin autoreceptor antagonist, (+)-AJ 76 (Casey et al., 1996), just as it appears to be a stronger locomotor stimulant (Svensson et al., 1986, Sonesson et al., 1994). These data support the contention that (−)-DS 121, a mild behavioral stimulant, may have some cocaine-mimetic properties. However, Clark et al. (1995) showed that (−)-DS 121 failed to produce a robust, dose-dependent substitution for cocaine (or *d*-amphetamine) in cueing experiments. In fact, (−)-DS 121 weakly antagonized the cueing properties of both stimulants. Additionally, results from other behavioral studies (Sonesson et al., 1994; Kling-Petersen et al., 1995b; Smith et al., 1996) suggest that (−)-DS 121 has weak, if any, positive reinforcing properties. (−)-DS 121 is, therefore, likely to be 'non-addictive' and its weak stimulant effects may actually de-

crease cocaine binging and craving in man. Finally, the cocaine blocking effects of (−)-DS 121 are physiologically reflected in the glucose metabolism studies.

Taken together, the data suggest that (−)-DS 121 may be beneficial in the pharmacotherapy of cocaine addiction. The mild stimulation would minimize cocaine craving, while the antagonist property would dampen the highly 'rewarding' effect which makes cocaine so 'addictive'. This contention is consistent with the findings of Smith et al. (1996), who demonstrated inhibition of cocaine self-administration by (−)-DS 121 in rats.

At this point, it is difficult to assess the importance of the dopamine D_3 preference of (−)-DS 121 in mediating its effects. The dopamine D_3 receptor is described as an autoreceptor as well as a postsynaptic receptor, with a high affinity for dopamine (Schwartz et al., 1993). It is interesting to note that the dopamine D_3 receptor is implicated in modulating mesolimbic dopamine release (Rivet et al., 1994) and synthesis (Aretha et al., 1995) an area also mainly affected by cocaine and associated with the brain reward pathway. However, the autoreceptor function of the dopamine D_3 receptor on dopamine release has been questioned (Waters et al., 1993b, but see Aretha et al., 1995), and selective dopamine D_3 antagonists have been shown to increase motor activity at doses that do not affect dopamine release (Waters et al., 1993b; Griffon et al., 1995).

Using microdialysis in freely moving rats, we demonstrated that (−)-DS 121 (15 mg/kg) increased levels of extracellular striatal dopamine. This finding is in agreement with previous studies reported by Sonesson et al. (1993). The increase in locomotor activity observed with this treatment also agrees with behavioral studies by Svensson et al. (1993), Sonesson et al. (1993), and Kling-Petersen et al. (1995b), who experimentally measured locomotor activity. In support of the 2-deoxyglucose autoradiography data, the dialysis experiment also revealed (−)-DS 121's stimulatory effect on extracellular dopamine. The combination of (−)-DS 121 and cocaine resulted in a synergistic increase in striatal dopamine release from approximately 300–800%. This phenomenon has earlier been described for the combination of dopamine receptor antagonists (such as haloperidol, (+)-UH 232 and (+)-AJ 76) and dopamine reuptake inhibitors such as GBR 12909 (Westerink et al., 1987; Waters et al., 1994). Interestingly, Waters et al. (1994) did not see this synergistic effect when combining the selective dopamine D_3 antagonist U-99194A with GBR 12909, which would suggest that the effects of (−)-DS 121 on dopamine release, when given alone or in combination with cocaine, are related to (−)-DS 121's blockade of release modulating dopamine D_2 autoreceptors.

A likely explanation for the different results obtained in the two assays may be the different synaptic sites experimentally monitored by the two studies. While autoradiography studies in general might give representative information about activities at both presynaptic and postsynaptic sites, microdialysis data represent activities solely at the presynaptic level, i.e., changes in neurotransmitter release or concentration within the synaptic cleft. Since cocaine exerts its direct effects at the presynaptic but its indirect effects at the postsynaptic sites, both approaches are still valid for the characterization of the pharmacological drug interaction between (−)-DS 121 and cocaine.

Indeed, (−)-DS 121, dependent on the dose administered, will affect presynaptic as well as postsynaptic sites. It is believed that (−)-DS 121 will preferentially antagonize dopamine autoreceptors. As a result of the subsequent increase in dopamine release, (−)-DS 121 will increase locomotor activity over a wide dose range. At higher doses, however, the drug also acts at postsynaptic dopamine receptors, thus being inhibitory on dopamine function. This inhibitory effect has been previously demonstrated by Svensson et al. (1993) and Waters et al. (1994), showing that a high dose of (−)-DS 121 (approximately 30–60 mg/kg s.c.) actually antagonizes *d*-amphetamine- or DP-5,6-ADTN-induced hyperactivity. Interestingly, however, the drug fails to reduce exploratory behavior or produce catalepsy. In line with this, it was shown that (−)-DS 121 displaces DP-5,6-ADTN in vivo from striatal binding sites, but to a lesser extent than haloperidol (Waters et al., 1994). Taken together, these findings further support the 'non-addictive', behavioral normalizing properties of (−)-DS 121, because the drug would have a self-limiting stimulant effect when reaching a dose that will be sufficient to act at both the pre- and postsynaptic sites. This may also explain the cocaine-blocking effects of (−)-DS 121 observed in the 2-deoxyglucose autoradiography study. After cocaine administration, increased dopamine and remaining antagonist will then compete for the postsynaptic sites.

In conclusion, (−)-DS 121 appears promising in counteracting cocaine-craving due to its mild stimulatory properties through preferential autoreceptor blockade, while the euphoria induced by cocaine may be blocked by (−)-DS 121's antagonist properties at postsynaptic dopamine receptors. Other preferential autoreceptor antagonists, such as (+)-AJ 76 and (+)-UH 232 (Svensson et al., 1986), share these properties (Piercey et al., 1992; Callahan et al., 1992; Richardson et al., 1993; Casey et al., 1996).

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