Nicotine- and methamphetamine-induced dopamine release evaluated with in-vivo binding of radiolabelled raclopride to dopamine D₂ receptors: comparison with in-vivo microdialysis data

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Abstract
The effect of substances which alter extracellular dopamine (DA) concentration has been studied by measuring changes in the binding of radiolabelled raclopride, a DA D₂ receptor ligand that is sensitive to endogenous DA. To better characterize the relationship between extracellular DA concentration and DA D₂ receptor binding of raclopride, we compared the changes of extracellular DA concentration (measured using in-vivo microdialysis) and in-vivo [³H]raclopride binding induced by different doses of methamphetamine (Meth) and nicotine, drugs that enhance DA release with and without blocking DA transporters (DATs), respectively, in rat striatum. Nicotine elicited a modest increase of striatal extrasynaptic extracellular DA, while Meth produced a marked increase of striatal extrasynaptic DA in a dose-dependent manner. There was a close correlation between the decrease in [³H]raclopride in-vivo binding and the increase in extrasynaptic DA concentration induced by both nicotine ($r^2 = 0.95$, $p < 0.001$) and Meth ($r^2 = 0.98$, $p = 0.001$), supporting the usefulness of the radiolabelled raclopride-binding measurement for the non-invasive assessment of DA release following interventions in the living brain. However, the linear regression analysis revealed that the ratio of percent DA increase to percent [³H]raclopride binding reduction was 25-fold higher for Meth (34.8:1) than for nicotine (1.4:1). The apparent discrepancy in the extrasynaptic DA–[³H]raclopride binding relationship between the DA-enhancing drugs with and without DAT-blocking property indicates that the competition between endogenous DA and radiolabelled raclopride takes place at the intrasynaptic rather than extrasynaptic DA D₂ receptors and reflects synaptic concentration of DA.

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Introduction
Investigating changes in synaptic dopamine (DA) concentration in response to pharmacological challenges in vivo in the human brain is a valuable tool in human neuropharmacology. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) neuroreceptor imaging techniques can be used to measure acute fluctuations in synaptic concentration of neurotransmitters. Competition between endogenous transmitters and radioligands for binding to neuroreceptors is the principle underlying this application. Rodent (Inoue et al. 1991; Kim et al. 1998; Ross & Jackson, 1989; Young et al. 1991), non-human primate (Carson et al. 1997; Dewey et al. 1993; Hartvig et al. 1997; Volkow et al. 1999) and human studies (Schlaepfer et al. 1997; Smith et al. 1998; Volkow et al. 1994; Wang et al. 1999) have suggested that changes attributable to synaptic DA can be shown

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using ligands such as $[^{11}C]/[^{3}H]$raclopride, a DA D$_2$ receptor radioligand that is sensitive to endogenous DA. PET studies using $[^{11}C]$raclopride have been successfully applied to characterize alterations of DA transmission in pharmacological and clinical conditions by measuring changes in the binding potential of the radioligand (Breier et al. 1997; Kim et al. 2004; Koepf et al. 1998; Volkow et al. 1997).

We have previously reported, in an abstract form (where the dose of nicotine was expressed as nicotine salt; Kim et al. 1998), that nicotine, a drug that enhances DA release without blocking DA transporters (DATs), induced a significant decrease in $[^{3}H]$raclopride in-vivo binding with only a modest increase in extrasynaptic extracellular DA concentration measured with in-vivo microdialysis. In contrast, the dose of amphetamine (a DA releaser having DAT-blocking property) associated with a similar decrease in $[^{11}C]$raclopride binding in monkeys elicited a much larger increase in extrasynaptic extracellular DA (Breier et al. 1997). Similar discrepancies have been shown between DA enhancers with and without DAT-blocking property in their ability to affect DA microdialysis and $[^{11}C]$raclopride binding potential measurements (Tsukada et al. 1999). Based on these observations, it has been suggested that the competition between endogenous DA and radioligand takes place at the intrasynaptic rather than extrasynaptic D$_2$ receptors and reflects the synaptic concentration of DA (Laruelle, 2000). Further validation studies directly comparing the extracellular DA–raclopride binding relationships associated with DA-enhancing drugs with and without DAT-blocking property are needed. In the present study we report different relationships between the effects of methamphetamine (Meth) and nicotine, drugs that enhance DA release with and without blocking DATs, respectively. Our data further support the notion that changes in radiolabelled raclopride binding are mediated by changes in synaptic rather than extrasynaptic DA concentration.

**Materials and methods**

**Animals**

Male Sprague–Dawley rats, weighing 280–320 g, were used for in-vivo microdialysis and in-vivo $[^{3}H]$raclopride binding experiments. The animals were group-housed in light-, temperature-, and humidity-controlled animal quarters with food and water available ad libitum. All procedures involving animal use were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

**Drugs**

(−)-Nicotine hydrogen tartrate was purchased from Sigma Chemical Co. (St Louis, USA). S(+)-Methamphetamine HCl was obtained from RBI (Natick, USA). $[^{3}H]$Raclopride (79.3 Ci/mmol) was purchased from NEN Life Science (Boston, USA).

**Surgery and microdialysis procedures**

Extrasynaptic extracellular DA concentrations were monitored in the striatum of freely moving rats following the different doses of nicotine (0.1–10 mg/kg s.c.; expressed as free base) and Meth (0.1–10 mg/kg i.p.) using in-vivo microdialysis. Rats were anaesthetized with sodium pentobarbital (50 mg/kg i.p.). Using aseptic techniques, a guide cannula (CMA/12, CMA Microdialysis, Solna, Sweden) aimed to terminate in the centre of the striatum (AP 1.0, L 3.2 from bregma; H 3.0 from dura; Paxinos & Watson, 1986) was stereotaxically implanted and attached to the skull using skull screws and dental cement. The cannula was then closed with a tight-fitting stainless-steel obturator. The microdialysis was performed in freely moving rats. Following 3-d recovery, a 4-mm microdialysis probe (CMA/12, CMA Microdialysis) connected via a dual liquid swivel to a syringe pump was inserted into the guide cannula and perfused with an artificial cerebrospinal fluid [145 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl$_2$, 1.0 mM MgCl$_2$, 2.0 mM Na$_2$HPO$_4$ (pH 7.4)] at a constant rate of 1.5 $\mu$L/min. Dialysate samples were collected during 20-min sampling intervals via outlet tubing connected to a microfraction collector (CMA Microdialysis). The position of the probes was verified by histological examination at the end of experiments.

**Analytical procedures**

The dialysate (injection volume 30 $\mu$L) was assayed for DA using HPLC coupled to an ESA Coulochem II 5200A electrochemical detection system (ESA Biosciences, USA) with an oxidation potential of +320 mV. The detector was equipped with a high performance analytical cell (ESA model 5014) which is tailored for use in microdialysis applications. The mobile phase was composed of 75 mM monobasic sodium phosphate, 0.1 mM EDTA, 1.4 mM octanesulfonic acid and 10% acetonitrile and adjusted to pH 3.2 with HPLC grade phosphoric acid. The separation of monoamine metabolites was performed on a Waters
Data are means ± S.E.M. (n = 4–6 for each group). *p < 0.05, **p < 0.005 vs. the control group.

Novo-Pak C-18 column (4 μm, 150 × 3.9 mm). The flow rate of the system was 1.0 ml/min. DA in dialysates was expressed as a percentage of three baseline samples collected immediately before drug treatment.

**In-vivo [3H]Raclopride-binding experiments**

Rats received different doses of nicotine (0.1–10 mg/kg s.c.; expressed as free base) or Meth (0.1–10 mg/kg i.p.) 20 min before injection of [3H]Raclopride. Control animals were injected with the same volume (0.5 ml) of saline. [3H]Raclopride (10 μCi in 0.5 ml saline) was injected intravenously into a tail vein, and 20 min later, the animals were sacrificed by cervical dislocation. Striata and cerebella were immediately dissected on ice, weighed and placed into glass vials. After digestion of the tissues with Soluene-350 (Packard Instrument Co., USA), 10 ml Formula 989 scintillator (Packard) were added and the samples counted in a liquid scintillation counter (Tri-Carb 2500 TR, Packard).

**In-vivo** binding of [3H]Raclopride to DA D2 receptors was expressed as the ratio of specific (striatal – cerebellar) to non-specific (cerebellar) uptake.

**Statistical analysis**

Values are expressed as mean ± S.E.M. Microdialysis data were analysed by one- and two-way (treatment × time) analysis of variance (ANOVA) with repeated measures, followed by Fisher’s PLSD post-hoc test. In-vivo [3H]Raclopride-binding data were analysed by one-way ANOVA and Fisher’s PLSD test. The relationship between extrasynaptic DA concentration and [3H]Raclopride binding was analysed with the Pearson linear correlation coefficient. A probability value of 0.05 was selected as the significance level.

**Results**

**Nicotine-induced changes in extrasynaptic extracellular DA concentration and in-vivo [3H]Raclopride binding**

Table 1 shows [3H]Raclopride uptake in the striatum and cerebellum, expressed as percentage of injected dose per gram of tissue (%ID/g tissue), as well as the (striatum – cerebellum)/cerebellum ratios at various doses of nicotine including control values. There was no significant difference in cerebellar [3H]Raclopride uptake in controls and animals treated with different doses of nicotine, while striatal uptake decreased significantly with increasing doses of nicotine within a limited dose range. Figure 1(a, c, e) shows the effect of nicotine on extrasynaptic DA concentration and its relationship with in-vivo binding of [3H]Raclopride in the striatum [expressed as the (striatum – cerebellum)/cerebellum ratio]. Subcutaneous injection of nicotine (0.1–10 mg/kg) elicited a modest increase of striatal extrasynaptic DA and a decrease of striatal [3H]Raclopride binding in a dose-dependent manner within a limited dose range. A dose of 1.8 mg/kg nicotine induced a 30.8 ± 8.3% increase in extrasynaptic DA.
baseline level at the peak time of 40 min after injection ($p < 0.01$). This dose of nicotine resulted in a $20.7 \pm 5.4\%$ decrease in $[^3]H$raclopride binding compared with control values ($5.272 \pm 0.360$ vs. $6.651 \pm 0.427$, $p < 0.05$). Increasing the dose of nicotine (3.5 mg/kg) led to a further increase in extrasynaptic DA by $51.2 \pm 9.1\%$ at the peak time of 40 min after injection ($p < 0.005$) and a decrease in $[^3]H$raclopride binding by
Table 2. [3H]Raclopride striatal and cerebellar uptake data at different doses of methamphetamine

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%ID/g = percent injected dose per gram tissue. Data are means ± s.e.m. (n = 4–6 for each group).

* p < 0.05, ** p < 0.0005, *** p < 0.00001 vs. the control group.

32.6 ± 3.2% (4.484 ± 0.215 vs. 6.651 ± 0.427, p < 0.005). When the nicotine dose was increased to 7 and 10 mg/kg, neither extrasynaptic DA nor [3H]raclopride binding were changed further compared with 3.5 mg/kg nicotine. Lower doses of nicotine (0.1 and 0.4 mg/kg) had no significant effect on either extrasynaptic DA or [3H]raclopride binding in the striatum. The decrease in [3H]raclopride binding was plotted against the increase in extrasynaptic DA concentration (Fig. 1c). There was a close correlation between the decrease in [3H]raclopride in-vivo binding and the increase in extrasynaptic DA concentration induced by nicotine (r² = 0.95, p < 0.001). The linear regression analysis revealed that 1% reduction of [3H]raclopride binding corresponds to a 1.4% increase in extrasynaptic DA concentration.

**Meth-induced changes in extrasynaptic extracellular DA concentration and in-vivo [3H]raclopride binding**

Table 2 shows [3H]raclopride uptake in the striatum and cerebellum, expressed as %ID/g tissue, as well as the (striatum – cerebellum)/cerebellum ratios at various doses of Meth including control values. There was no significant difference in cerebellar [3H]raclopride uptake among controls and animals treated with different doses of Meth, while striatal uptake decreased significantly with increasing doses of Meth. Figure 1(b, d, f) shows the effect of Meth on extrasynaptic DA concentration and its relationship with in-vivo binding of [3H]raclopride in the striatum [expressed as the (striatum – cerebellum)/cerebellum ratio]. In contrast to nicotine, intraperitoneal injection of Meth (0.1–10 mg/kg) produced a marked increase of striatal extrasynaptic DA in a dose-dependent manner. However, it induced a dose-dependent decrease in striatal [3H]raclopride binding only with a similar magnitude to that attained by nicotine. Doses of 0.1–10 mg/kg Meth elicited 118–1371% increases in extrasynaptic DA over baseline level at the peak time of 40 min after injection (p < 0.01–0.0001), while these doses of Meth caused only 5.9–42.1% decreases in [3H]raclopride binding compared with control values (5.504 ± 0.123–3.388 ± 0.369 vs. 5.848 ± 0.269, p < 0.05–0.00001). The decrease in [3H]raclopride binding was plotted against the increase in extrasynaptic DA concentration (Fig. 1f). There was a close correlation between the decrease in [3H]raclopride in-vivo binding and the increase in extrasynaptic DA concentration induced by Meth (r² = 0.98, p = 0.001). The linear regression analysis revealed that 1% reduction of [3H]raclopride binding corresponds to a 34.8% increase in extrasynaptic DA concentration.

**Discussion**

We found a close correlation between the decrease in [3H]raclopride in-vivo binding and the increase in extrasynaptic DA concentration induced by both nicotine and Meth, supporting the usefulness of the radioligand-binding measurement in providing non-invasive assessment of DA release.

If the competition between endogenous DA and radioligand occurs mostly at non-synaptic DA D₁ receptors, microdialysis measurements of extrasynaptic DA and decrease in [3H]raclopride binding would be related across pharmacological challenges. However, our data demonstrate that the extrasynaptic DA-[3H]raclopride binding relationship is quite different following Meth and nicotine, drugs that enhance DA release with and without blocking DATs, respectively. A large increase in extrasynaptic DA concentration induced by Meth was associated with a relatively small effect on [3H]raclopride binding. The ratio of percent DA increase to percent binding reduction was 34.8:1 for Meth. This is consistent with
the ratios of amphetamine-induced DA release to [\textsuperscript{3}H]iodobenzamide (44:1; Laruelle et al. 1997) and [\textsuperscript{11}C]raclopride (44–64:1; Breier et al. 1997) binding potential reductions. In contrast, as revealed in the present study, the ratio of percent DA increase to percent [\textsuperscript{3}H]raclopride binding reduction was 1.4:1 for nicotine. As a consequence, a similar reduction in [\textsuperscript{3}H]raclopride binding (~30%) was observed following nicotine (3.5 mg/kg) and Meth (5 mg/kg) challenges associated with 51% and 826% increase in extrasynaptic DA, respectively. Extrasynaptic DA reflects intrasynaptic DA following DAT blockers such as amphetamine and Meth, but not following drugs like nicotine, which enhances DA release without blocking DAT (Laruelle, 2000). Hence, the apparent discrepancy in the extrasynaptic DA-[\textsuperscript{3}H]raclopride binding relationship following Meth and nicotine indicates that the competition between endogenous DA and radiolabelled raclopride takes place at the intrasynaptic rather than extrasynaptic DA D_2 receptors and reflects synaptic concentration of DA. It may be that extrasynaptic D_2 receptors are less sensitive to endogenous DA, conceivably due to their configuration in the agonist low-affinity state. Similarly, a PET study combined with microdialysis reported that at similar level of [\textsuperscript{11}C]raclopride binding-potential decrease, the magnitude of extracellular DA increase following direct DA enhancers (DAT blockers: GBR 12909 and Meth) was much larger than following indirect enhancers (a muscarinic cholinergic antagonist benztropine and a 5-HT_2A antagonist ketanserin; Tsukada et al. 1999). These results also are consistent with a predominant intrasynaptic location of the effect measured with PET.

Grace (1991, 1993) proposed the term ‘phasic release’ to characterize the intrasynaptic release of DA elicited by cell firing, and the term ‘tonic release’ to characterize the low level of extrasynaptic release that is independent of cell firing. In this sense, endogenous competition studies using radiolabelled benzamides are of great use for the assessment of phasic activity of dopaminergic neurons in neuropsychiatric disorders such as schizophrenia (Abi-Dargham et al. 2000), attention deficit hyperactivity disorder (Grace, 2001) and cognitive conditions associated with novel and rewarding stimuli (Kim et al. 2004; Koeppe et al. 1998; Schultz, 2000).

In the present study, the magnitude of reductions of in-vivo [\textsuperscript{3}H]raclopride binding by nicotine and Meth did not exceed 50% of control values. These results are consistent with PET and SPECT studies in non-human primates and humans that indicate that only up to 50% of the in-vivo binding of [\textsuperscript{11}C]raclopride and [\textsuperscript{123}I]iodobenzamide is affected by psychostimulant challenges (Abi-Dargham et al. 1998; Boojt et al. 1997; Breier et al. 1997; Dewey et al. 1993; Laruelle et al. 1995, 1997; Volkow et al. 1994, 1997, 1999; Wang et al. 1999). This ceiling effect may be explained by several factors, such as DA D_2 receptors configured in a state of low affinity for the agonist DA, D_2 receptors occupied by baseline levels of endogenous DA, and extrasynaptic D_2 receptors that might be less exposed to changes in DA release compared with receptors located in the synaptic cleft (Laruelle, 2000). Narendran et al. (2005) showed that a large proportion (70–80%) of DA D_2 receptors configured in a state of high affinity for agonists in vivo using the D_2 receptor agonist [\textsuperscript{3}H]N-propyl-norapomorphine (NPA) and the D_2 receptor antagonist [\textsuperscript{11}C]raclopride in PET studies in baboons. This may explain the vulnerability of D_2 radiotracers to competition by endogenous DA. They also showed that at a tracer dose, [\textsuperscript{11}C]NPA binding potential was almost exclusively associated with high-affinity state receptors, suggesting that the in-vivo binding of agonist radiotracer [\textsuperscript{11}C]NPA is more vulnerable to competition by endogenous DA than that of the antagonist radiotracer [\textsuperscript{11}C]raclopride.

Tsukada et al. (2002) found that nicotine at ~0.06 mg/kg or 0.18 mg/kg did not cause [\textsuperscript{11}C]raclopride displacement in the striatum of conscious monkeys, but a small effect was seen in the same animals under isoflurane anaesthesia. Marenco et al. (2004) also reported that nicotine doses ranging from 0.01 to 0.06 mg/kg produced only 5% reduction in [\textsuperscript{11}C]raclopride binding potential in monkeys under isoflurane anaesthesia. These non-human primate PET data are consistent with our findings that doses of nicotine <0.4 mg/kg did not cause changes in [\textsuperscript{3}H]raclopride binding in rats. Moreover, the observed magnitude of changes in [\textsuperscript{3}H]raclopride binding after Meth administration is comparable to non-human primate PET data (Marenco et al. 2004; Tsukada et al. 2002).

As shown in the present study, it is obvious that Meth causes a much larger increase in extrasynaptic DA than nicotine. This is probably due to the different mechanisms of action of the two drugs. Meth blocks DATs and causes presynaptic DA release (Kuczenski & Segal, 1989), while nicotine presumably increases burst firing of dopaminergic neurons for a short period of time (Grenhoff et al. 1986; Murase et al. 1993). In this study, systemic injection of nicotine produced only a modest increase in striatal DA output. It is known that the nigrostriatal system is less sensitive to the effects of nicotine than the mesolimbic system (Di Chiara & Imperato, 1988; Imperato et al. 1986;
Mereu et al. (1987). We found that nicotine-induced striatal DA output reached a plateau at a dose of nicotine higher than 3.5 mg/kg. This may be due to several factors, such as possible curvilinear relationship between the nicotine dose administered and the magnitude of nicotinic acetylcholine receptor occupancy, rapid desensitization of nicotinic acetylcholine receptors (Grady et al., 1994), nicotinic acetylcholine receptor modulation of DAT function and thereby enhancement by nicotine of DA clearance (Middleton et al., 2004), and distinct allosteric states of the nicotinic acetylcholine receptor with different affinities for agonists (Krauss et al., 2000).

This study has limitations. First, we determined [3H]raclopride-specific binding associated with nicotine and Meth using striatal and cerebellar uptake at the single time-point after tracer administration, not by measuring kinetics of ligand-receptor binding. Pharmacological interventions that produce significant changes in synaptic DA concentration frequently affect physiological parameters such as body temperature, regional cerebral blood flow, peripheral clearance, or non-specific binding of radiotracers, which are known to affect radiotracer uptake. This raises the importance of model-based methods that are resistant to variations in these physiological parameters for appropriate evaluation of the effect of pharmacological challenges on receptor availability. Nevertheless, in the present study, doses of either nicotine or Meth did not alter cerebellar [3H]raclopride uptake at the time of euthanasia compared with control values, while striatal uptake decreased with increasing doses of nicotine and Meth. Therefore, we believe that the nicotine- and Meth-induced decrease in [3H]raclopride-specific binding represents a reduction in binding potential, not resulting from challenge-induced changes in physiological parameters. Second, we could not compare the extrasynaptic DA and DA D2 receptor-binding data obtained from the same animals. In-vivo imaging studies combined with microdialysis would allow the comparison within the same animals.

Conclusion

The close correlation between the decrease in [3H]raclopride in-vivo binding and the increase in extrasynaptic DA concentration supports the usefulness of the radiolabelled raclopride-binding measurement for the non-invasive assessment of DA release following pharmacological and non-pharmacological interventions in the living brain. However, we further demonstrated that the extrasynaptic DA–[3H]raclopride binding relationship is quite different following Meth and nicotine, drugs that enhance DA release with and without blocking DATs, respectively. The ratio of percent DA increase to percent [3H]raclopride binding reduction was 25-fold higher for Meth (34.8:1) than for nicotine (1.4:1). The apparent discrepancy between the drugs with and without DAT-blocking property indicates that the competition between endogenous DA and radiolabelled raclopride takes place at the intrasynaptic rather than extrasynaptic DA D2 receptors and reflects synaptic concentration of DA.

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Statement of Interest

None.

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