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Original article

Coupled intracerebral microdialysis and electrophysiology for the assessment of dopamine neuron function *in vivo*

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ABSTRACT

Introduction: The central dopaminergic system is involved in the pathophysiology of several neuropsychiatric disorders. Intracerebral microdialysis and electrophysiology provide two powerful techniques to investigate dopamine (DA) function and the mechanism of action of psychotropic drugs *in vivo*. **Methods**: Here, we developed a protocol allowing the combined measurement of neurochemical and electrical activities of the nigrostriatal and mesoaccumbens DA pathways, by coupling *in vivo* microdialysis and electrophysiology in the same isoflurane-anesthetized animal. DA neuron firing rate and burst firing were measured in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), whereas extracellular levels of DA and its main metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were monitored in the striatum and the nucleus accumbens (NAc). The validity of the protocol was assessed using various drugs known to modify DA neuron activity *in vivo*. **Results**: The peripheral administration of the DA-D_2 agonist quinpirole decreased SNc DA neuron firing rate and burst firing, as well as DA and DOPAC outflow in the rat striatum. Opposite effects were observed after the peripheral administration of the DA-D_2 antagonist haloperidol. In rats and mice, the peripheral administration of cocaine elicited a decrease in VTA DA neuron firing rate and burst firing, and an increase in accumbal DA outflow, paralleled by a reduction in DOPAC outflow. **Discussion**: The obtained results, confirming previous electrophysiological and microdialysis studies, demonstrate that this protocol provides a suitable method for the study of DA neuron function and the mechanism of action of psychotropic drugs in the living brain of both rats and mice.
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1. Introduction

During the last thirty years, a sizable body of literature has demonstrated that intracerebral microdialysis is a powerful technique to investigate the mechanism of action of psychotropic drugs and their impact on neuronal function in the brain of living animals (Westerink & Cremers, 2007). In this context, a large number of studies have been devoted to the central dopaminergic system, in keeping with its role in the pathophysiology of several neuropsychiatric disorders, such as depression, schizophrenia, Parkinson's disease and drug addiction (Di Chiara & Bassareo, 2007; Dunlop & Nemeroff, 2007; Howes & Kapur, 2009; Schapira, 2009). Extensive pharmacological investigations have shown that *in vivo* monitoring of extracellular levels of dopamine (DA) and its main metabolites provides an accurate biochemical index to evaluate midbrain DA neuron activity (Di Chiara, 1990). Indeed, microdialysis studies have given major contribution to the knowledge of the regulatory neurochemistry underlying the control of DA release and metabolism *in vivo*, and the mechanism of action of centrally acting drugs (Di Chiara, 1990; Sharp & Zetterström, 2007).

Single unit recording of DA neuron activity represents another powerful approach to investigate DA neuron function *in vivo* (Bunney, Chiodo, & Grace, 1991). With respect to intracerebral microdialysis, monitoring of DA neuron firing rate and burst firing provides important complementary information, especially when investigating the impact of psychotropic drugs on DA neuron function. Indeed, although burst firing is tightly associated to neurotransmitter release (Chergui, Suaud-Chagny, & Gonon, 1994; Manley, Kuczenski, Segal, Young, & Groves, 1992), drug-induced DA release is not always paralleled by an increase in DA neuronal firing, as, depending on the drug mechanism of action, increase in DA release can occur independently from increased DA neuron firing. For instance, whereas morphine- and haloperidol-stimulated DA release is associated with an increase in DA neuron firing (Di Chiara & Imperato, 1988; Lucas et al., 2001; Matthews & German, 1984; Porras, Di Matteo, De Deurwaerdère, Esposito, & Spampinato, 2002), the outflow of DA induced by cocaine

Abbreviations: DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; NAc, nucleus accumbens; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; PLSD, protected least significance difference.

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or amphetamine is associated with a decrease in DA neuron firing (Pitts & Marwah, 1988; Porras et al., 2002). Indeed, at variance with morphine or haloperidol, whose stimulatory effects on DA release are thought to be a consequence of an increase in DA neuron firing (Di Chiara & Imperato, 1988; Matthews & German, 1984), cocaine and amphetamine enhance DA outflow by blocking or inverting DA transporter function, respectively (Cadoni, Pinna, Russi, Consolo, & Di Chiara, 1995; Carboni, Imperato, Perezzani, & Di Chiara, 1989; Pitts & Marwah, 1988; White, 1990). Thus, measurement of the electrical activity of DA neurons is a key parameter to take into account when studying the mechanism of action of psychotropic drugs. These considerations point out that joint monitoring of electrical and neurochemical activity of DA neurons in the same animal would provide a powerful tool for investigating DA neuron function *in vivo*. Interestingly, this approach was used in a previous study (Lee, Abercrombie, & Tepper, 2004), assessing the regulatory influence of GABAergic neurons on the nigrostriatal DA pathway activity in anesthetized rats. However, the paper by Lee et al. (2004) does not provide a pharmacological validation of the employed methodology, making difficult to generalize its use to the study of ascending DA pathway activity *in vivo*.

Hence, on the basis of the work by Lee et al. (2004) and our previous experience with microdialysis and electrophysiology, the present study was aimed at developing and validating a new protocol for the combined measurement in the same animal of both neurochemical and electrical activities of the nigrostriatal and mesoaccumbens DA pathways, by coupling *in vivo* microdialysis and extracellular electrophysiology approaches in isoflurane-anesthetized rats or mice. DA neuron firing rate and burst firing were measured at the level of the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), whereas extracellular levels of DA and its main metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were monitored at the level of the striatum and the nucleus accumbens (NAc), the sites of projection of SNc and VTA DA neurons respectively (Ungerstedt, 1971). To validate the efficacy of the protocol, we assessed the responsiveness of subcortical DA pathways to the peripheral administration of various drugs (the DA-D_2 receptor agonist quinpirole, the DA-D_2 receptor antagonist haloperidol and cocaine), whose effects on DA neuron firing, release and metabolism are well described in previous electrophysiological (Gao, Lee, King, & Ellinwood, 1998; Lucas et al., 2001; Pitts & Marwah, 1988; White, 1990) and microdialysis studies (Di Chiara & Imperato, 1988; Koeltzow, Austin, & Vezina, 2003; Lucas et al., 2001; Porras et al., 2002; Navailles, De Deurwaerdère, Porras, & Spampinato, 2004). In a first group of experiments, focusing on the nigrostriatal DA pathway, we studied the effects of quinpirole or haloperidol on SNc DA neuron firing and striatal DA and DOPAC outflow in rats. In a second experiment, in keeping with the key role of the mesolimbic DA system in mediating the rewarding properties of drugs abused (Di Chiara & Bassareo, 2007; Hyman, Malenka, & Nestler, 2006), we assessed the effect of cocaine on VTA DA neuron firing and accumbal DA and DOPAC outflow in rats. Finally, this latter experiment was also performed in mice, to validate a possible use of the proposed protocol for studies in transgenic mice, which are widely used in the field of neuroscience research.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (Charles River, Saint Germain sur l'Arbresle, France) weighing 330–350 g, and C57/Bl6J male mice (Charles River, Saint Germain sur l'Arbresle, France) weighing 29–35 g were used. Animals were housed at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a 12 h light/dark cycle (dark from 20:00 h) and had free access to water and food. Animal use procedures conformed to the International European Ethical Standards (86/609-EEC) and the French National Committee (*décret* 87/848) for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Coupled in vivo microdialysis and electrophysiology

2.2.1. General procedures

Surgery and perfusion procedure were performed according to a previously described protocol (Lee et al., 2004; Leggio et al., 2009) with minor modifications, to allow concomitant electrophysiological and microdialysis monitoring. Briefly, animals were anesthetized using a 2% mixture of isoflurane/air, and a catheter was inserted into the femoral (rats) or the jugular (mice) vein for intravenous drug administration. Thereafter, animals were placed in a stereotaxic frame (David Kopf Instruments, Phymep, Paris, France) equipped with a nose mask for constant delivery of the gas anesthesia (2% isoflurane during surgery, 1.5% isoflurane during electrophysiology and microdialysis experiment), and their rectal temperature was monitored and maintained at 37 ± 1 °C by a heating pad (CMA 150, Carnégie Medecin, Phymep). After exposing the dorsal skull, two holes were drilled to allow the subsequent ipsilateral implantation of a microdialysis probe (CMA/11 or CMA/7 for rats or mice, respectively, 240 μm outer diameter, Cuprophan; Carnegie Medicin, Phymep) in the right striatum (probe: 4 mm long) or the medio-ventral part of the right NAc corresponding to the shell subdivision (probe: 2 mm or 1 mm long for rats or mice, respectively), and a recording electrode (glass micropipette TW150F-4, 2–3 μm outer diameter, WPI-Europe, Aston Stevenage, UK) in the right SNc or VTA (see Fig. 1A). For the placement of microdialysis probes, the stereotaxic coordinates (in mm relative to bregma) were as follows: rat striatum: anteroposterior (AP) = +0.7, lateral (L) = 2.8, ventral (V) − 7.4; rat NAc: AP = +1.7, L = 1, V = −8; mouse NAc: AP = +1.4, L = 0.6, V = −5, according to the atlas of either Paxinos and Watson (1986) or Franklin and Paxinos (1997) for rats or mice, respectively. For the placement of the recording electrodes, the stereotaxic coordinates (in mm relative to bregma and cortical surface) were as follows: rat SNc: AP = −5.4–5.8, L = 1.8–2.5, V = −7.0–8.0; rat VTA: AP = −5.4–5.8, L = 0.4–0.8, V = −7.0–8.5; mouse VTA : AP = −3.3–3.5, L = 0.3–0.6, V = −3.5–4.3 (Franklin & Paxinos, 1997; Paxinos & Watson, 1986). After implantation, the probes were perfused at a constant rate (2 μl/min or 1.1 μl/min for rats or mice, respectively) by means of a microperfusion pump (CMA 111, Carnegie Medicin, Phymep) with artificial cerebrospinal fluid (aCSF) containing (in mM): 154.1 Cl^-, 147 Na^+, 2.7 K^+, 1 Mg^{2+}, and 1.2 Ca^{2+}, adjusted to pH 7.4 with 2 mM sodium phosphate buffer. Perfusion was then maintained for 2 h (1 h in mice experiments), to allow the stabilization of DA and DOPAC levels in the perfusates. Thereafter, single unit recording of DA neuronal firing and monitoring of DA and DOPAC extracellular levels were started (see Fig. 1B). On the one hand, dialysates were collected on ice every 15 or 20 min for rats (30 μl) or mice (22 μl) respectively, and immediately analyzed to determine the baseline values of DA and DOPAC extracellular levels, defined by three consecutive samples in which DA and DOPAC content varied by less than 10% (Leggio et al., 2009). On the other hand, the search of DA neurons was started for electrophysiological recording. Since the time needed to detect and identify DA neurons is random, the time searching was adjusted in each experiment so that firing baseline was obtained at the same time than that of the DA and DOPAC baseline values. In particular, once a DA neuron with stable firing rate was found, the recording electrode downhill was stopped waiting the stabilization of DA and DOPAC contents in the dialysates. Thereafter, DA neuron firing rate was recorded for 3–5 min to obtain the firing baseline, defined by a variation of less than 10% of the average frequency discharge of the DA neuron.

2.2.2. DA neuron recording

Recording of DA neuron firing was performed according to a methodology routinely used in the laboratory (Ambroggi et al., 2009). Single

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