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EFFECTS OF INTRANASALLY APPLIED DOPAMINE ON BEHAVIORAL ASYMMETRIES IN RATS WITH UNILATERAL 6-HYDROXYDOPAMINE LESIONS OF THE NIGRO-STRIATAL TRACT

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Abstract—Due to its lipophobic properties, dopamine is unable to cross the blood–brain barrier following systemic application. However, recently it has been demonstrated that, when applied directly via the nasal passages in the rat, dopamine exerts neurochemical and behavioural action, including increases of dopamine in striatal subregions, antidepressive-like action, and increased behavioral activity. These effects could potentially be mediated by exogenous dopamine acting as a direct agonist at postsynaptic dopamine receptors. However, it is also possible that intranasally applied dopamine acts indirectly via the modulation of the activity of dopaminergic cell bodies. To approach this question, the present study used rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal tract, as these lesions lead to pharmacologically stimulated behavioural asymmetries which are specific for direct and indirect dopamine agonists. We found that 7 days of repeated treatment with intranasal dopamine induced a sensitization of the turning response to amphetamine, but not to apomorphine. Furthermore, intranasal dopamine dose-dependently increased the use of the forepaw ipsilateral to the 6-OHDA-lesioned side of the brain. These results suggest that intranasally administered dopamine acts via an indirect mechanism of action, putatively by increasing the release of endogenous dopamine in the brain.

Key words: intranasal dopamine, 6-hydroxydopamine, behavioural asymmetry, forepaw use, turning behaviour.

Dopamine (DA), due to its hydrophilic properties cannot cross the blood–brain barrier following ordinary systemic administration. However, recent evidence has indicated that DA, when applied intranasally (i.n.), may be able to enter the brain, and exert neurochemical as well as behavioural effects. Nasal DA application in rats increased extracellular DA in dorsal and ventral striatum (De Souza Silva et al., 2008), it had antidepressive-like action and increased behavioral activity (Buddenberg et al., 2008), and in an animal model of attention deficit hyperactivity disorder, attenuated activity levels and deficits in attention (Ruocco et al., in press). Basically, the transport of drugs from the nose to the brain could be achieved via the olfactory epithelium, via the olfactory nerve, or via the systemic circulation from the nasal mucosa (Illum, 2000). Dahlin et al. (2001) reported that i.n. administered [^{3}H]DA was found not only in the olfactory bulb, but also in the cerebrum, which raises the possibility that exogenous DA could act as a direct agonist at postsynaptic DA receptors. However, it is also possible that an indirect mechanism mediates the neurochemical and behavioural effects of i.n. applied DA, i.e. exogenously applied DA could stimulate DAergic cell bodies, thus leading to an increase of extracellular DA in the striatum (De Souza Silva et al., 2008). This question remains to be determined. The unilateral 6-hydroxydopamine (6-OHDA) lesion model provides a way to differentiate between the possible alternative mechanisms of action of i.n.-applied DA, because direct and indirect DA agonists have well known and clearly distinguishable behavioural effects following such a lesion (Schwarting and Huston, 1996). Indirect DA agonists, like amphetamine, act on the non-lesioned side and induce behavioural asymmetries favouring the side ipsilateral to the lesion, while direct agonists, like apomorphine, act on supersensitive DA receptors on the lesioned side and induce behavioural asymmetries favouring the side contralateral to the lesion (Schwarting and Huston, 1996). If i.n. applied DA acts as a direct agonist in the dorsal striatum, it should be expected to induce apomorphine-like behaviour, while amphetamine-like behaviour should be evident if it acts indirectly via accumulation of endogenous DA in the dorsal striatum on the non-lesioned side (De Souza Silva et al., 2008). Therefore, the present experiment investigated the effects of different doses of i.n. applied DA on behaviours induced by a unilateral 6-OHDA-lesion of the nigrostriatal tract.

EXPERIMENTAL PROCEDURES

All experiments were conducted in conformity with the Animal Protection Law of the Federal Republic of Germany and with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to limit the number of animals used and to minimize discomfort.

Animals

Male Wistar rats (Tierversuchsanlage, University of Düsseldorf, Germany) weighing between 300 and 330 g were used. They were housed four animals per cage under standard laboratory

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Abbreviations: DA, dopamine; i.n., intranasally; 6-OHDA, 6-hydroxydopamine.

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conditions, with a reversed 12-h light/dark rhythm (light on from 19:00 to 07:00 h) and food and water provided *ad libitum*. After arrival, they were given at least two weeks to adapt to the reversed light/dark cycle before they went into surgery.

Surgery

Animals were anaesthetized with a mixture of 0.9 ml/kg Ketavet (containing 100 mg/ml ketamine; Pharmacia and Upjohn, Germany) and 0.2 ml/kg Rompun (containing 20 mg/ml xylazine; Bayer, Germany), and 1 ml/kg Rimadyl (containing 5 mg/kg carprofen; Pfizer, Karlsruhe, Germany) was injected s.c. to prevent post-operative pain. The rats were placed in a Kopf stereotaxic frame, the scalp was cut and retracted to expose the skull. A hole was drilled above the left substantia nigra. Dopaminergic lesions were performed by injection of 6-OHDA (8 μg in 1 μl saline containing 0.1% ascorbic acid) into the left substantia nigra (AP: −4.3 mm, ML: −2.0 mm, DV: −8.0 mm; relative to bregma and skull surface). Following the injection, the cannula was left in place for 4 min. Finally, the wound was sutured and disinfected with a 70% ethanol solution. On the day following surgery, behavioural measurements were begun (Fornaguera et al., 1995; Schwarting et al., 1991).

Experimental procedures

Testing was carried out on 11 subsequent days in an open field (48×48×48 cm) situated in a sound attenuating box (110×70×70 cm). A camera was mounted 66 cm above the open field, and connected to a VCR and a personal computer running a variation of the VIAS software (Schwarting et al., 1993) provided by Dr. Jay-Shake Li, which automatically recorded turning behaviour. Illumination was provided by two red light bulbs (luminous density on floor level ~8 lx). On the first 7 days, animals in different groups received an i.n. application of DA (vehicle n=9, 1.5 n=6, 3.0 n=9, 6.0 n=7 mg/kg, one-half dose into each nostril) and were placed into the open field for 60-min. On days 8 and 10 following surgery, each animal received an i.n. application of vehicle (DopaMat; Mattern Pharmaceuticals AG, Switzerland) and on days 9 and 11, an injection of apomorphine (1.0 mg/kg in saline; s.c.) or amphetamine (1.5 mg/kg in saline; i.p.). The order of apomorphine and amphetamine injections was counterbalanced, so that half of the animals received apomorphine on day 9 and amphetamine on day 11 and the other half of the animals received amphetamine on day 9 and apomorphine on day 11. For days 1–7, post hoc analyses of rearing against the wall supported by the left or by the right forepaw were performed for the first 30 min of each test-trial using the EthoVision (Noldus, Wageningen, The Netherlands) video tracking system.

Neurochemical analysis

After the experiment, the animals were deeply anaesthetized by CO_2 and decapitated and the brain was excised and placed into cold 0.5 M perchloric acid. The left and right ventral striatum, dorsal striatum, and medial prefrontal cortex were dissected separately, homogenised, centrifuged, filtered, and stored at −80 °C until analysis (De Souza Silva et al., 1997). The samples were analysed for their contents of noradrenaline (NE), serotonin (5-HT) and DA by means of HPLC-EC. The column was an ET 125/2, Nucleosil 120-5, C-18 reversed phase column (Macherey & Nagel, Germany) perfused with a mobile phase composed of 75 mM NaH_2PO_4, 4 mM KCl, 20 μM EDTA, 1.5 mM sodium dodecylsulfate, 100 μl/l diethylamine, 12% methanol and 12% acetonitrile adjusted to pH 6.0 using phosphoric acid. The electrochemical detector (Intro, Antec, The Netherlands) was set at 500 mV vs. an ISAAC reference electrode (Antec, Leyden, The Netherlands) at 30 °C.

Statistics

Tissue levels of monoamines were analysed by paired samples t-tests, comparing values from the lesioned side with values from the intact side separately for each brain area. Analyses of turning behaviour were performed by three way ANOVAs with the factors treatment (0, 1.5, 3.0, 6.0 mg/kg), side (ipsiversive, contraversive), and day (treatment days 1–7). These were followed by two-way ANOVAs with the factors treatment and day considering the orientation of turning. Turning behaviour on the amphetamine and apomorphine treatment days was analysed by two-way ANOVAs with the factors treatment-group (0, 1.5, 3.0, 6.0 mg/kg i.n. DA) and side, followed by one-way ANOVAs with the factor treatment-group on left and right turns in combination with LSD post hoc tests. Furthermore, the time course of turning and frequency of rearing against the wall supported with the left or right forepaw following i.n. DA were analysed. Ipsiversive and contraversive turning for experimental days 1–7 was analysed by two-way ANOVAs with the factors treatment and time. Wall-supported rearing was analysed by three-way ANOVAs with the factors treatment, paw (ipsilateral to the lesion, contralateral to the lesion) and time. This was followed by separate two-way ANOVAs for the right and left paw with the factors treatment and time, and by LSD post hoc tests.

RESULTS

Postmortem neurochemistry

In the lesioned hemisphere there was a 91% decrease of DA tissue levels (P=0.01) in the dorsal striatum, and there were also trends towards a decrease of 5-HT (36.5%; P=0.051) and NA (73%; P=0.076). There were no significant effects of the 6-OHDA lesion on tissue levels of DA, 5-HT or NA in the nucleus accumbens (P>0.05), while in the medial prefrontal cortex there was a significant decrease of NA (54%; P=0.019) on the lesioned side and no significant effect on levels of DA and 5-HT (P>0.05). Tissue contents of neurotransmitters were not related to the DA treatment group, as treatment-group comparisons within brain areas did not yield any significant differences (data not shown).

Turning behaviour

As expected the animals with unilateral 6-OHDA lesions showed ipsiversive turning behaviour during the seven days following surgery. Furthermore, this behavioural asymmetry was shown for the whole duration of the experiment. However, turning behaviour was not consistently influenced by prior i.n. DA treatment.

The treatment×side×day ANOVA on turning behaviour (Fig. 1A–D) showed a significant effect of side [F(1,27)=90.276; P<0.001], but no effect of treatment or day, no interaction between treatment and day, treatment and side, and no three-way interaction (P>0.05). Subsequent two-way ANOVAs conducted separately for ipsiversive and contraversive turning, did not indicate any significant main effects and no interaction between treatment and day for turns towards the side of the lesion. For turns away from the lesioned side there was a significant effect of day [F(6,162)=2.324; P=0.035], but no effect of treatment and no interaction (P>0.05).