Changes in extracellular dopamine in the rat globus pallidus induced by typical and atypical antipsychotic drugs

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Abstract

Typical antipsychotic drugs with a high extrapyramidal motor side-effects liability markedly increase extracellular dopamine in the caudate-putamen, while atypical antipsychotic drugs with a low incidence of extrapyramidal motor side-effects have less pronounced stimulating actions on striatal dopamine. Therefore, it has been suggested that the extrapyramidal motor side-effects liability of antipsychotic drugs (APD) is correlated with their ability to increase extracellular dopamine in the caudate-putamen.

The globus pallidus (GP) is another basal ganglia structure probably mediating extrapyramidal motor side-effects of typical antipsychotic drugs. Therefore, the present study sought to determine whether extracellular dopamine in the globus pallidus might be a further indicator to differentiate neurochemical actions of typical and atypical antipsychotic drugs. Using in vivo microdialysis we compared effects on pallidal dopamine induced by typical and atypical antipsychotic drugs in rats. Experiment I demonstrated that systemic administration of haloperidol (1 mg/kg; i.p.) and clozapine (20 mg/kg; i.p.) induced a significant pallidal dopamine release to about 160 and 180% of baseline, respectively. Experiment II revealed that reverse microdialysis of raclopride and clozapine using a cumulative dosing regimen did not stimulate extracellular dopamine in the globus pallidus if low (1/200 M) or intermediate (10 and 100 M) concentrations were used. Only at a high concentration (1000 M), raclopride and clozapine induced a significant pallidal dopamine release to about 130 and 300% of baseline values, respectively. Thus, effects of typical and atypical antipsychotic drugs on pallidal dopamine were similar and thus, may not be related to their differential extrapyramidal motor side-effects liability. Furthermore, the finding that reverse microdialysis of raclopride over a wide range of concentrations did not stimulate pallidal dopamine concentrations tentatively suggests that pallidal dopamine release under basal conditions is not regulated by D2 autoreceptors.

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1. Introduction

Clinical application of antipsychotic drugs (APD) is accompanied by extrapyramidal motor side-effects such as parkinsonism. Drugs with a high liability for extrapyramidal motor side-effects such as haloperidol (Seeman and Van Tol, 1994) were defined as "typical" APD, while drugs being largely devoid of extrapyramidal motor side-effects, e.g. clozapine (Coward, 1992; Meltzer et al., 1999), were termed as "atypical" APD.

A large number of studies have been devoted to differentiate the sites and mechanisms of actions of typical and atypical APD. One hypothesis suggests that typical APD have a higher extrapyramidal motor side-effects liability because they generally induce a more pronounced dopamine (DA) D2 receptor occupancy than atypical APD (e.g. Farde et al., 1988; Farde and Nordstrom, 1992; Farde et al., 1992; Kapur et al., 2000; Mukherjee et al., 2001). Alternatively, the higher motor side-effects liability of typical APD as compared to atypical APD may be related to their specific actions on DA release in different forebrain areas. All APD stimulate DA release in the caudate-putamen, nucleus accumbens and prefrontal cortex (e.g. Moghaddam and Bunney, 1990) probably reflecting a feedback response to the blockade of DA receptors (e.g. Carlson and Lindqvist, 1963; Chodro and Bunney, 1983; Walters and Roth, 1976). However, the regional pattern differs as atypical APD have more prominent actions on extracellular DA in the nucleus accumbens and prefrontal cortex than in the caudate-putamen, while typical APD have stronger effects in the caudate-putamen (Moghaddam and...
Thus, differential neurochemical accpallidal GABA levels have been linked with parkinsonism. Systemic administration of clozapine (Chapman and See, 2001) increased extracellular GABA in the GP, but decreased after systemic administration of haloperidol, but increased after systemic administration of clozapine (Chapman and See, 2001). In line with this notion, immunohistochemical studies using induction of immediate early genes as a marker of neuronal activity showed that atypical APD produced less prominent c-fos expression in the caudate-putamen than typical APD (e.g., Nguyen et al., 1992; Robertson and Fibiger, 1992; Bunser and Deutch, 2002). Most studies addressing possible sites mediating extrapyramidal motor side-effects have been focused on the caudate-putamen. However, there are other basal ganglia candidate structures down-stream of the caudate-putamen such as the globus pallidus (GP) which might mediate extrapyramidal motor side-effects. Recent studies revealed that extracellular GABA in the GP is increased after systemic administration of haloperidol, but decreased after systemic administration of clozapine (Chapman and See, 1996). In functional models of the basal ganglia, increased pallidal GABA levels have been linked with parkinsonism (Albin et al., 1989). Thus, differential neurochemical actions of typical versus atypical APD on pallidal GABA might be related to their differential extrapyramidal motor side-effects liability (See and Berglind, 2001; See et al., 2002). Apart from GABA, DA in the GP plays a prominent role in mediating motor effects of APD as well. The GP receives a DAergic innervation arising from substantia nigra pars compacta neurons (Fallon and Moore, 1978; Lindvall and Bjorkland, 1979) and pallidal DA release is of neuronal origin (Hauber and Fuchs, 2000; Fuchs and Hauber, 2004). Systemic and intra-GP infusion of D2 receptor antagonists increased expression of c-fos in the GP (Raskin and Marshall, 1997; Marshall et al., 2001). Furthermore, intra-GP infusion of DA receptor antagonists produced akinesia (Costall et al., 1972; Hauber and Lutz, 1999) suggesting that changes in pallidal DAergic transmission might contribute to extrapyramidal motor side-effects induced by typical APD.

The present study sought to determine whether extracellular DA in the GP may be an indicator to differentiate ne rochemical actions of atypical and typical APD. Using in vivo-microdialysis we investigated whether the typical APD haloperidol and the atypical APD clozapine given systemically differ in their effects on extracellular pallidal DA in a similar way as shown previously in the caudate-putamen. Furthermore, we analysed by reverse microdialysis whether the effects of a typical and an atypical APD on pallidal DA are mediated by local actions.

2. Material and methods

The animal experiments described in this study were conducted according to the German Law on Animal Protection and were approved by the proper authorities in Stuttgart, Germany. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.1. Subjects

Male CD rats (Charles River, Sulzfeld, Germany) were housed in groups up to five animals in transparent Macrolon® cages (type IV) (35 cm × 21 cm × 30 cm; Ebeco, Castrop-Rauxel, Germany) before surgery. Temperature (20 ± 2°C) and humidity (50 ± 10%) were kept constant in the animal house and a 12:12-h light-dark schedule with lights on at 6:00 h was maintained. Rats were given ad libitum access to water. Food (standard maintenance chow, Altromin, Lage, Germany) was restricted to 15 g per day and animal.

2.2. Experiment I: Systemic administration of clozapine and haloperidol

Rats received intraperitoneal injections of either 1 mg/kg haloperidol (N = 5), 20 mg/kg clozapine (N = 5) or saline (N = 4) in the respective volume. Injections were made at the beginning of a microdialysis sampling period.

2.3. Experiment II: Reverse microdialysis of clozapine and raclopride

Rats were perfused with increasing concentrations (1, 10, 100 and 1000 μM) of either clozapine (N = 5) or raclopride (N = 5) for 60 min following perfusion of artificial cerebrospinal fluid (aCSF) without drug for one additional sample.

2.4. Stereotaxic surgery

Animals were anaesthetized with sodium pentobarbital (60 mg/kg, i.p.) (Sigma-Aldrich, Taufkirchen, Germany) following pretreatment with atropine sulphate (0.5 mg/kg, i.p.) (Sigma-Aldrich, Taufkirchen, Germany) and secured in a Kopf stereotaxic apparatus (Kopf Instruments, Tujunga, USA). For experiment I, animals weighing 230–360 g were implanted unilaterally with intracranioid guide cannulae (MAB 6.14 IC, Microbiotech/se, Stockholm, Sweden) aiming to the GP at the following coordinates (Paxinos and Watson, 1986): AP = −1.1 mm, L 2.5 mm and V = 4.5 mm (from dura). For experiment II, animals weighing 240–370 g were implanted with intracranial guide cannulae (CMA/12, CMA, Solna, Sweden) at the same coordinates as above. After the surgery rats were housed individually in Macrolon® cages (type III; 37 cm × 21 cm × 30 cm; Ebeco, Castrop-Rauxel, Germany) with raised solid-walled lids. Each rat was given at least one week to recover from surgery.

2.5. Microdialysis

Microdialysis was performed in the home cage of the animal with the lid replaced by a metal frame bearing a
counterbalanced arm with the swivel assembly. Dual channel liquid swivels, either Tsumura TCS2-23 (Pronexus, Skåholmen, Sweden) or Inotech low torque swivels (375/D22/QM, Inotech Laboratory, Plymouth Meeting, USA) were used. Attachment to the swivel was achieved by a spring tether connected directly to the head mount via a self-made plug.

A microdialysis probe either MAB 6.14.2 (Microbiotech, Stockholm, Sweden, experiment I) or CMA/12 (CMA, Solna, Sweden, experiment II) with an exposed membrane length of 2 mm was inserted through the guide cannula at least 7 h before sampling of the first baseline value and perfused with aCSF (145 mM Na\(^+\), 2.5 mM K\(^+\), 2.4 mM Ca\(^{2+}\) [experiment I] or 1.2 mM Ca\(^{2+}\) [experiment II] and 0.9 mM Mg\(^{2+}\)) delivered by a CMA/100 microdialysis pump (CMA, Solna, Sweden) at 2.0 µl/min. aCSF for experiment I contained 3 µM nomifensine, a catecholamine reuptake inhibitor. Samples were collected every 30 min. Basal levels were determined 60 min before drug administration in each experiment.

2.6. Drugs

In experiment I, sterile saline (Fresenius, Bad Homburg, Germany), haloperidol (Haldol-Janssen, Janssen, Neuss, Germany), and clozapine (Tocris, Ellisville, USA) were used for systemic administration. Clozapine was diluted with 0.1 M HCl to a final concentration of 10 mg/ml and used for systemic administration. Clozapine, haloperidol (Haldol-Janssen, Janssen, Neuss, Germany), and clozapine (Tocris, Ellisville, USA) were used. Attachment to the swivel was achieved by a spring tether connected directly to the head mount via a self-made plug.

2.7. Analytical procedure

Dialysates were analysed for DA, DOPAC and HVA using HPLC with electrochemical detection. The mobile phase used in experiment I consisted of 2 g/l sodium acetate, 5 g/l citric acid, 200–600 mg/l l-heptanesulfonic acid, 110 mg/l Na\(_2\)EDTA and 12.5% (v/v) methanol with pH at 3.9 before methanol addition. In experiment II, the mobile phase had a higher concentration of methanol (18.5%, v/v) and HSA (2000 mg/l). Minor modifications in the concentrations of HSA and methanol were made to optimise DA peak separation, if necessary. The HPLC apparatus consisted of a Flux Rheos 2000 pump (Flux Instruments, Basel, Switzerland), a refrigerated CMA/200 autosampler (CMA, Solna, Sweden), a Nucleosil C18 column (Bischoff, Leonberg, Germany; 5 µm particles, length × i.d. 125 mm × 3 mm) and a dual electrode BAS LC4C amperometric detector (Bioanalytical Systems, Lafayette, USA) with the electrode potential set to 600 mV at high gain to quantify DA at 700 mV at a low gain to measure the metabolites. Filter setting was 0.1 Hz. The separation was performed at room temperature and sample run time was less than 7 min. The detection limit of DA in a standard solution was about 2 pg per injection or lower.

2.8. Reconstruction of probe location

After the experiments, animals were euthanised by an overdose of sodium pentobarbital, the brains removed, fixed for at least 2 h in formalin and immersed in 30% (w/v) sucrose for several days. Cryosections (60 µm) were taken and stained with cresyl violet. Only data from animals with correct probe location, i.e. most of the exposed dialysis membrane located within the GP, were evaluated (Fig. 1).

2.9. Data expression and statistics

Data are expressed as mean percentages of control values (± standard error of the mean, S.E.M.). The average concentration of three samples before drug administration (not corrected for probe recovery) was taken as control and set to 100%. Data were analysed by one-way ANOVA for repeated measurements followed by Least square differences (LSD) post hoc test. The last baseline sample was taken as reference. An α-level of P < 0.05 was regarded to represent statistical significances. Statistical analysis were made with Statistica 5.5 (StatSoft, Tulsa, USA).

3. Results

3.1. Basal dialysate DA levels

In experiment I, basal dialysate DA concentrations were 27.7 ± 4.4 pg/60 µl in saline-treated animals (N = 4),
3.2. Experiment I: Systemic administration of clozapine and haloperidol

3.2.1. Effects on DA

As shown in Fig. 2 administration of clozapine (20 mg/kg, i.p.) produced a significant increase ($F_{1,6} = 4.57, P = 0.0031$) of dialysate DA to about 180% of baseline, administration of haloperidol (1 mg/kg, i.p.) a significant increase to about 160% of baseline ($F_{1,6} = 48.24, P < 0.00001$). Statistical analysis further revealed a significant effect in the saline group ($F_{1,6} = 3.29, P < 0.023$), but there were no significant differences of samples after saline treatment relative to the baseline value ($T = 0$ min) as revealed by post hoc LSD-test.

3.2.2. Effects on DOPAC and HVA

Administration of clozapine and haloperidol produced increases in dialysate DOPAC ($F_{1,6} = 5.68, P < 0.00006$ and $F_{1,6} = 12.21, P < 0.00001$, respectively) and HVA ($F_{1,6} = 2.59, P = 0.044$ and $F_{1,6} = 2.72, P < 0.037$, respectively) levels. In saline controls no significant changes in DOPAC ($F_{1,6} = 0.56, P = 0.76$) or HVA ($F_{1,6} = 0.50, P = 0.797$) were observed (Fig. 3).

3.3. Experiment II: Reverse microdialysis of clozapine and raclopride

3.3.1. Effects on DA

Raclopride and clozapine significantly elevated dialysate DA levels ($F_{1,11} = 3.19, P = 0.003$ and $F_{1,11} = 14.51, P < 0.00001$, respectively) (Fig. 4). Post hoc LSD
Fig. 3. Effect of systemic administration of clozapine (20 mg/kg i.p.), haloperidol (1 mg/kg i.p.) or saline (1 ml/kg i.p.) on pallidal extracellular DOPAC (upper panel) and HVA (lower panel) concentrations. *, clozapine; §, haloperidol indicate significant differences relative to the last baseline sample (T = 0 min) (P < 0.05, ANOVA for repeated measurements followed by post hoc LSD test).

3.3.2. Effects on DOPAC and HVA

Dialysate DOPAC levels were increased significantly by raclopride or clozapine at the highest concentration (1 mM) \( (F_{1,11} = 3.11, P = 0.0056 \) and \( F_{1,11} = 2.37, P = 0.028 \), respectively). Clozapine at the highest concentration (1 mM) had also significant effects on dialysate HVA \( (F_{1,11} = 2.37, P = 0.028) \), while raclopride had no significant effects \( (F_{1,11} = 1.62, P = 0.126) \) (Fig. 5).

4. Discussion

The present study demonstrates that a typical and an atypical APD can increase the extracellular DA concentration in the GP if administered systemically. However, local administration of a typical and an atypical ADP by reverse...
microdialysis did not stimulate extracellular DA concentration in the GP except at a very high concentration.

4.1. APD effects after systemic administration

Doses of clozapine at least 10 times higher than haloperidol were considered to be equipotent and generally used to compare neurochemical effects of both drugs (e.g. Meltzer et al., 1994; Pehek and Yamamoto, 1994; Gray and Connick, 1998; Ichikawa and Meltzer, 2000). Therefore, we administered a dose of clozapine 20-fold higher than haloperidol. Results demonstrate that both drugs induced a significant pallidal DA release with an onset at the initial post-administration sample. Thus, a typical and an atypical APD given systemically in about equipotent doses can elicit a similar pallidal DA efflux. In addition, clozapine and haloperidol elevated pallidal DOPAC indicating drug effects on pallidal DA metabolism as extracellular DOPAC is considered to be a marker for cytoplasmatic DA synthesis (Zetterstrom et al., 1988). Likewise, both drugs increased dialysate HVA levels which might reflect the fact that most extracellular DOPAC is rapidly converted to HVA by the catechol-O-methyltransferase (Cumming et al., 1992).

A large number of studies revealed that haloperidol is more effective in increasing extracellular DA in the caudate-putamen than clozapine (e.g. Pehek and Yamamoto, 1994; Volonte et al., 1997) probably reflecting a feedback response to the blockade of D2 receptors (e.g. Carlsson and Lindqvist, 1963; Chiodo and Bunney, 1983; Walters and Roth, 1976). However, some studies did not provide evidence for a clear-cut dissociation of the effects of clozapine and haloperidol on striatal DA (Moghaddam and Bunney, 1990; Gray and Connick, 1998). Moreover, typical APD produce much higher expression of immediate early genes, e.g. c-fos, in the caudate-putamen than atypical APD (Nguyen et al., 1992; Robertson and Fibiger, 1992; Bubser and Deutch, 2002). Therefore, it has been hypothesized that the high liability of typical APD for extrapyramidal motor side-effects might be related to their prominent actions on D2 receptors in the caudate-putamen (Nordstrom et al., 1993; Moghaddam and Bunney, 1990; Volonte et al., 1997; Seeman, 2001). By analogy, we expected that haloperidol has stronger stimulating effects on pallidal DA than clozapine. However, our data provide no evidence in support of this notion. Thus, effects of typical and atypical APD on pallidal DA may not account for their differential motor side-effects liability. This does not argue against the GP as a possible site of such effects as systemic administration and intra-GP infusion of haloperidol increased, while clozapine decreased pallidal GABA, effects that might be associated with their respective extrapyramidal motor side-effect profiles (Chapman and Sec, 1996; See and Berglind, 2001; See et al., 2002; but see Drew et al., 1990). However, the differential motor side-effects liability of typical and atypical APD may not be solely related to their preferential action on striatal DA. Positron emission tomography (PET) binding studies revealed that high D2 receptor occupancies (>80%) are necessary to produce extrapyramidal motor side-effects (e.g. Farde et al., 1992; Kapur et al., 2000) which are more easily achieved with typical than atypical APD (e.g. Farde et al., 1988; Farde and Nordstrom, 1992; Mukherjee et al., 2001).
Fig. 5. Effect of reverse microdialysis of clozapine or raclopride in increasing concentrations from 1 to 1000 μM on pallidal extracellular DOPAC (upper panel) and HVA (lower panel) concentrations. *: clozapine indicate significant differences relative to the last baseline sample (T = 0 min) (P < 0.05, ANOVA for repeated measurements followed by post hoc LSD test).

4.2. APD effects after reverse microdialysis into the GP

Comparisons of results from experiments I and II have to take into account that microdialysis conditions differ in some aspects, e.g. the perfusion fluid in experiment II contained a lower calcium concentration, no nomifensine and different microdialysis probes were used. Furthermore, in experiment II we used the selective D2 antagonist raclopride instead of haloperidol as typical APD for reverse microdialysis due to its superior solubility in aCSF with neutral pH. However, raclopride and haloperidol bind in vivo almost exclusively to D2 receptors (Hall et al., 1989), thus, allowing at least qualitative comparisons of results from both experiments performed here.

Results of experiment II revealed that clozapine and raclopride failed to induce a significant pallidal DA release at concentrations up to 100 μM. However, at concentration of 1 mM, clozapine produced a prominent, raclopride a moderate albeit significant pallidal DA release. The fact that pallidal perfusion of the highly selective D2 receptor antagonist and typical APD raclopride (Seeman and Van Tol, 1994) stimulated extracellular DA only at a very high concentration suggests that a pallidal D2 receptor blockade does not account for the increase of pallidal DA induced by systemic
lates pallidal DA efflux due to disinhibition. In line with this prominent decrease of pallidal GABA, which in turn stimulates might be one of several possible mechanisms to extracellular GABA release (Cooper and Stanford, 2001) are located on strato-palidal terminals (Mansour et al., 1990; Weiner et al., 1991); however, there is no evidence for the existence of presynaptic D2 receptors located on nigropallidal terminals. Thus, our findings point to the view that D2 autoreceptors might not be involved in regulation of extracellular pallidal DA. Alternatively, the DA tone acting on D2 autoreceptors might be very low under basal conditions, because the GP receives only a “sparse” DA innervation (Arluison et al., 1984). In line with this notion, pallidal D2 receptor blockade did not decrease pallidal GABA levels (See and Berglind, 2001) despite the presence of presynaptic D2 receptors on striatopallidal terminals (Mansour et al., 1990). The GP bears D3 receptors (Larson and Ariano, 1995; Gurevich and Joyce, 1999) which display an unusually high affinity to DA (Levesque et al., 1992) and regulate DA release and synthesis (Sotnikova et al., 2001). Thus, D3 receptors could act as pallidal autoreceptors, however, their ultrastructural localization and role in regulation of extracellular DA in the GP is yet unknown. Clozapine failed to stimulate DA efflux in the GP except at the highest concentration implicating that pallidal actions are unlikely to account for the increase of pallidal DA observed after systemic clozapine. Clozapine displays high affinities to multiple receptors, including 5HT2A receptors, D1 receptors, D4 receptors, muscarinic receptors and α-adrenoceptors (Coward, 1992). From the present data, it is not possible to delineate which of these receptor mechanisms might mediate the pallidal DA increase induced by the highest concentration of clozapine. Recent data show that reverse microdialysis of 100 μM clozapine reduced pallidal GABA levels (See and Berglind, 2001). Extracellular GABA could be derived from neuronal and glial sources (Campbell et al., 1993) and microdialysis techniques do not easily allow to distinguish them. However, GABA microdialysis in a wide range of concentrations. This tentatively suggests that pallidal DA release under basal conditions is not regulated by D2 autoreceptors, which may be explained: (i) by a general lack of autoreceptors in the GP, or, (ii) by a DA tone too low to stimulate autoreceptors under basal conditions. Furthermore, our results demonstrate that systemic administration of a typical and an atypical APD stimulated pallidal DA and DA metabolism with a similar magnitude. Therefore, effects of typical and atypical APD on pallidal DA seem to be similar and thus, may not be related to their differential extrapyramidal motor side-effects liability.

4.3. Conclusions

Taken together, the present data show that raclopride did not stimulate pallidal DA efflux when administered by reverse microdialysis in a wide range of concentrations. This tentatively suggests that pallidal DA release under basal conditions is not regulated by D2 autoreceptors, which may be explained: (i) by a general lack of autoreceptors in the GP, or, (ii) by a DA tone too low to stimulate autoreceptors under basal conditions. Furthermore, our results demonstrate that systemic administration of a typical and an atypical APD stimulated pallidal DA and DA metabolism with a similar magnitude. Therefore, effects of typical and atypical APD on pallidal DA seem to be similar and thus, may not be related to their differential extrapyramidal motor side-effects liability.

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