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Effects of risperidone, clozapine and haloperidol on extracellular recordings of substantia nigra reticulata neurons of the rat brain

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Abstract

Risperidone has proven to be effective as an antipsychotic drug and has fewer extrapyramidal side-effects than classic neuroleptics. In addition to its dopamine D_2 receptor antagonistic properties, this antipsychotic agent is a potent 5-HT_2 receptor antagonist. The atypical antipsychotic, clozapine, also possesses both dopamine D_2 and 5-HT_2 receptor affinity next to affinities for other receptors. To gain an insight in the consequences for basal ganglia activity of treatment with these atypical neuroleptics vs. typical neuroleptics, the effects of cumulative doses of risperidone, clozapine and haloperidol on the firing rate of substantia nigra reticulata neurons were studied. Extracellular recordings were performed in chloralhydrate-anaesthetized male Wistar rats. Both risperidone (50–3200 μg/kg i.v.) and clozapine (100–6400 μg/kg i.v.) dose dependently decreased substantia nigra reticulata activity maximally to 70% of the basal activity. With both treatments, a dose of 800 μg/kg was significantly effective. In contrast, haloperidol (12.5–800 μg/kg i.v.) gradually induced a slight increase in substantia nigra reticulata activity, which was identical to the substantia nigra reticulata activity after saline treatment. Therefore, these results indicate that typical and atypical neuroleptics affect differentially the output of the basal ganglia in the substantia nigra reticulata. To evaluate the involvement of 5-HT_2 receptors in the effect of risperidone, the 5-HT_2 receptor agonist, quipazine (0.5 mg/kg i.p.), was administered 15 min preceding risperidone treatment. A 4-fold higher dose of risperidone was needed to significantly affect the substantia nigra reticulata firing rate. Thus, the 5-HT_2 component of the effect of risperidone is, at least partly, responsible for the difference in effect on substantia nigra reticulata neurons in comparison to haloperidol.

Keywords: Substantia nigra reticulata; Antipsychotic; Schizophrenia; Basal ganglion; Electrophysiology; Dopamine; 5-HT (5-hydroxytryptamine, serotonin)

1. Introduction

Risperidone has proven to be effective as an antipsychotic drug with fewer extrapyramidal side-effects than classic neuroleptics, like haloperidol (e.g., Roose et al., 1988; Castelao et al., 1989; Chouinard et al., 1993). This classifies the drug as atypical. Unlike typical neuroleptics, which possess predominant dopamine D_2 receptor antagonistic properties, risperidone has far greater affinity for 5-HT_2 receptors than for dopamine D_2 receptors (Leysen et al., 1988). Clozapine, the classic atypical antipsychotic without extrapyramidal side-effects (Claghorn et al., 1987; Kane et al., 1988), also displays a high ratio of 5-HT_2 to dopamine D_2 receptor affinity (e.g., Fink et al., 1984; Meltzer, 1989). It has therefore been proposed that integrated interference with both serotonin and dopamine systems by blockade of 5-HT_2 and dopamine D_2 receptors characterizes an atypical antipsychotic activity profile (Meltzer, 1989). Support for this suggestion comes from both animal and human studies. In rats, haloperidol-induced catalepsy could be alleviated by 5-HT_2 receptor antagonists, like ritanserin, and worsened by quipazine, a 5-HT_2 receptor agonist (Fuenmayor and Vogt, 1979; Balsara et al., 1979; Hicks, 1990; Bethany et al., 1993). In schizophrenic patients, ritanserin reduces haloperidol-induced extrapyramidal side-effects (Gelders et al., 1986; Gelders, 1989).

The neural basis for the serotonin-dopamine interaction that is thought to contribute to the unique therapeutic profile of atypical antipsychotics is far from clear. It has been shown (White and Wang, 1983) that atypical neuroleptics preferentially act on the mesolimbic-cortical dopaminergic system (dopamine neurons projecting from

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the ventral tegmental area to the nucleus accumbens and the prefrontal cortex), which is associated with an antipsychotic action (Mathysse, 1973; Stevens, 1973; Snyder, 1976), while typical neuroleptics affect the mesolimbic-cortical as well as the striatal dopaminergic system (dopaminergic neurons projecting from the substantia nigra to the striatum), the latter being held responsible for the occurrence of extrapyramidal side-effects (Baldessarini and Tarsy, 1980). These differential effects on the mesolimbic-cortical vs. the striatal dopaminergic system, however, do not explain the mechanism of action of the different classes of neuroleptics, since the dopaminergic systems are part of the basal ganglia, in which their role is clearly modulatory in character (for review, see DeLong, 1990). Projections of these two dopaminergic systems are not confined to limbic vs. striatal postsynaptic structures (Nauta and Domesick, 1984; Gerfen et al., 1985). Therefore, an insight in the consequences of specific changes in dopaminergic activity at postsynaptic structures should contribute to the understanding of how therapeutic and side-effects of neuroleptics are mediated. On the other hand, there may be involved, in addition to effects mediated via the dopaminergic systems mentioned, blockade of 5-HT_2 or dopamine D_2 receptors on other neuronal pathways. Thus, elucidation of the implications of treatment with atypical and typical neuroleptics for basal ganglia functioning in general should broaden our insight into the mechanism of action of these neuroleptics.

The substantia nigra reticulata, together with the internal segment of the globus pallidus, constitute the major output structures of the basal ganglia. Cortically derived information is processed through the basal ganglia nuclei and relayed via these inhibitory output pathways to thalamic and brainstem nuclei (Uno et al., 1978; Chevalier et al., 1981; Hikosaka and Wurtz, 1983). The dopaminergic neurons are in a position to affect this flow of information through the basal ganglia (for review, see DeLong, 1990). Thus, the activity level of the substantia nigra reticulata neurons reflects the overall response of changes in activity within basal ganglia structures. Therefore, in this study, intravenously administered cumulative doses of risperidone and clozapine were compared to haloperidol for their effects on the firing rate of substantia nigra reticulata neurons. For risperidone, the involvement of 5-HT_2 receptors was evaluated by pretreatment with the 5-HT_2 receptor agonist, quipazine.

2. Materials and methods

2.1. Animals and drugs

Male albino Wistar rats (270–320 g; CDL Groningen, Netherlands) were used for the experiments and were kept on a 12-h light/dark cycle with lights on at 6 a.m. The rats had free access to food and water.

The drugs used for the experiments were risperidone (kindly donated by Janssen Pharmaceuticals, Beerse, Belgium), clozapine, quipazine (Research Biochemicals International, Natick, MA, USA) and haloperidol (Sigma, St. Louis, MO, USA). Risperidone, clozapine and haloperidol were dissolved in a 0.9% saline solution, pH 5, and administered intravenously (i.v.). Quipazine was dissolved in 0.9% saline solution and injected intraperitoneally (i.p.).

2.2. Surgery and experimental procedures

The rats were anaesthetized with chloralhydrate (400 mg/kg i.p.) and anaesthesia was maintained during the total duration of the experimental procedure by additional i.p. injections of chloralhydrate every 30 min. A piece of polyethylene tubing (PE 10), which was filled with a 0.9% saline solution and connected to a syringe containing the identical solution, was inserted into the jugular vein approximately 3 cm in the direction of the heart. Subsequently, rats were placed in a stereotaxic frame (Kopf). Body temperature was maintained at 37°C by means of a heating pad. A hole was drilled above the substantia nigra reticulata at the coordinates AP -5.3 and ML ± 2.2 from the bregma (Paxinos and Watson, 1982). Two quad-Teflon-coated stainless-steel microwire electrodes (California Fine Wire, Grover City, CA, USA) were soldered into a Microtech (Boothwyn, PA, USA) miniature connector strip (GF-4). The diameter of the wire was 50 μm at the tip with no insulation and 100 μm with insulation. The two electrodes were positioned parallel, were equally long and approximately 1.5 mm apart. One of the electrodes was used for positioning at the substantia nigra reticulata coordinates, the other electrode was used, when needed in case of undefined noise, as an indifferent electrode. The electrodes were slowly lowered using a microdrive until substantia nigra reticulata single-unit activity was observed at the electrode used for positioning, providing the indifferent electrode was free of neuronal signal. Only occasionally, when single-unit activity could not be obtained, was multi-unit activity recorded. The activity pattern characteristic of substantia nigra reticulata neurons, i.e., a firing rate of 15–40 Hz and biphasic action potentials of 0.5–0.7 ms duration (Guyenet and Aghajanian, 1978; Waszczak et al., 1980), was encountered approx. 7.0–8.1 mm below the dura. The amplified electrophysiological signals (AM Systems, Everett, USA) were continuously monitored on an oscilloscope and audiospeaker. Computerized data acquisition (Spike2 CED, Cambridge, UK) served for on-line template identification performed on the rough data. The data were recorded both as individual events and as ratemeter record of the number of events per 10-s bin.

When stable baseline activity had been obtained for at least 30 min, data acquisition took place during a 5-min baseline period (from $t = -5$ to $t = 0$ min) followed by a recording period during which cumulative i.v. injections, at $t = 0$, 5, 10, 15, 20, 25 and 30 min of risperidone (50–3200

μg/kg), clozapine (100–6400 μg/kg), haloperidol (12.5–800 μg/kg) or saline, respectively, were administered. The doses were doubled for each step, yielding a total of seven doses in a volume of 1.6 ml 0.9% saline pH 5 solution. The total recording period was 40 min. Regarding the number of rats, $n = 4$ was used for saline injections, $n = 5$ for risperidone, $n = 5$ for clozapine and $n = 4$ for haloperidol. Each rat underwent the entire procedure of seven cumulative drug injections. In the pretreated group, the baseline period (from $t = -20$ to $t = -15$ min) was followed by an i.p. injection of quipazine at $t = -15$ min, followed by cumulative i.v. injections of risperidone or saline, respectively at $t = 0$, 5, 10, 15, 20, 25 and 30 min. The total recording period for the latter experiments was 55 min. $n = 4$ was used for quipazine and saline injections and $n = 5$ for quipazine and risperidone. Each rat underwent the entire procedure of one pretreatment injection followed by seven cumulative drug injections. For all experiments, the 10-s bin counts during the 5-min pretreatment interval were averaged and this value was used as 100% baseline activity. The means of the 10-s bin count data were calculated for 5-min intervals throughout the experiments and these values were converted to percent of baseline. Each rat was used for only one experiment.

2.3. Statistics

The data were analysed using a two-way analysis of variance with repeated measures, followed by the Scheffé multiple comparisons test for comparison of differently treated groups with the saline groups.

2.4. Histology

Upon completion of the experiments, the recording site of the recording electrode was marked by passing current

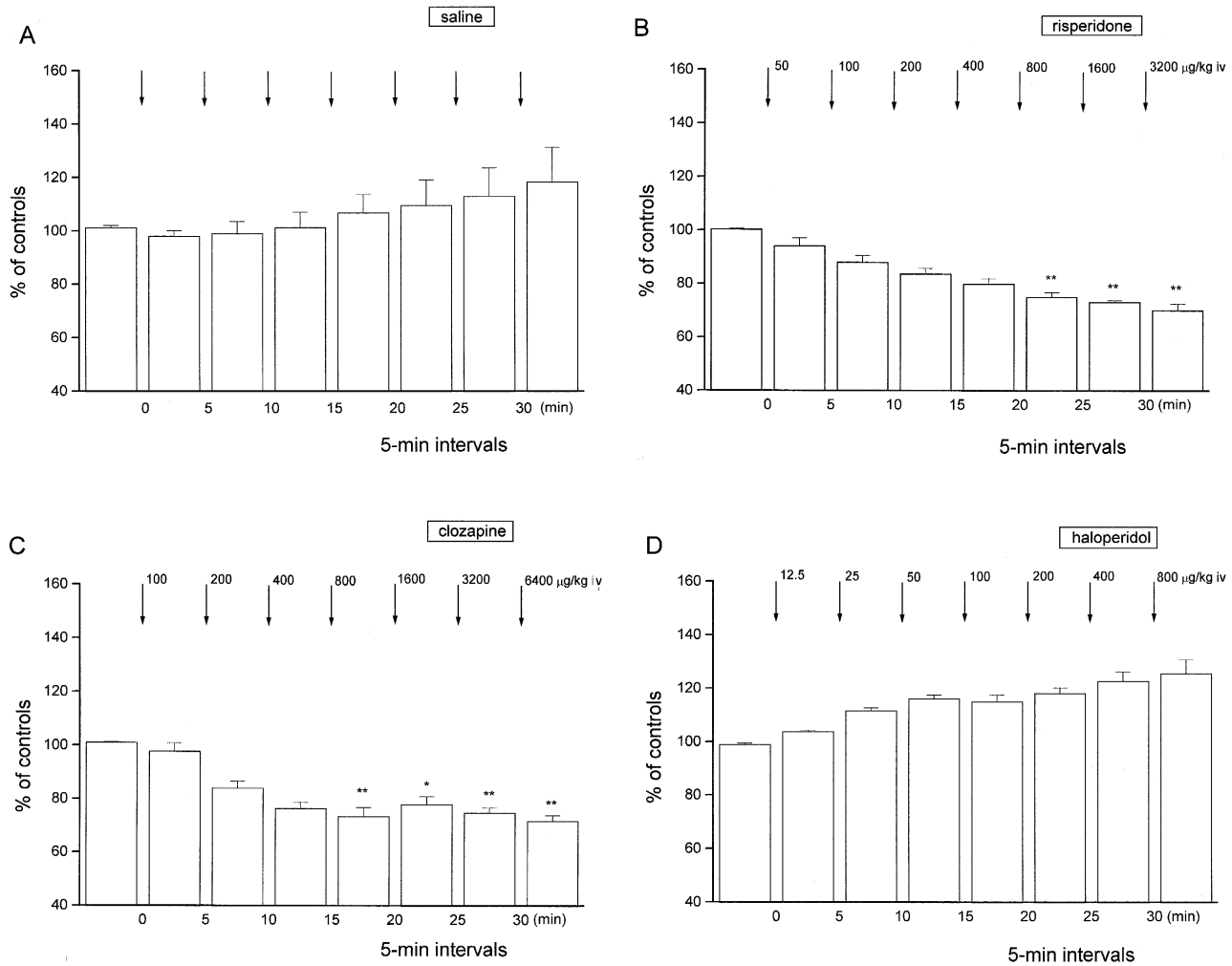

Fig. 1. Effects of cumulative injections of (A) saline ($n = 4$), (B) risperidone (50–3200 μg/kg i.v.; $n = 5$), (C) clozapine (100–6400 μg/kg i.v.; $n = 5$) and (D) haloperidol (12.5–800 μg/kg i.v.; $n = 4$), respectively, on substantia nigra reticulata extracellular recordings. Data are expressed as percentages of baseline activity, determined from the average of successive 10-s bin counts obtained over a 5-min interval, shown in the first column. The other columns represent the average activity over 5-min intervals, which were preceded by cumulative i.v. injections of the drugs. All values are means ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ vs. saline.

through the electrode (50 μA for 10 s). The animals were then perfused with a 4% formaldehyde solution which was lethal. The brain was removed and stored in a 5% potassium ferrocyanide-4% formaldehyde solution. The stored brain was eventually frozen and slices, 16 μm, were taken throughout the substantia nigra reticulata to identify the placement of the electrode. Data were included in the analyses only if the electrode was located in the medial part of the substantia nigra reticulata.

3. Results

3.1. Effect of risperidone, clozapine and haloperidol on substantia nigra reticulata neuronal activity

Upon saline injection, in volumes identical to those used for the drug injections, the substantia nigra reticulata activity gradually increased to 120% of baseline activity after the last dose was administered (Fig. 1A). As the rat had received an additional chloralhydrate injection 10 min before the start of the experiment, the gradual slight increase in activity most likely reflects the level of anaesthesia, since the activity level of the substantia nigra reticulata neurons increases slightly when anaesthesia becomes less deep. In one rat, during the experiment, at $t = 10$ min, an additional chloralhydrate dose was given to verify this explanation, which indeed decreased the activity level in the substantia nigra reticulata to baseline values. This rat was not included in the data shown in Fig. 1A.

Risperidone (50–3200 μg/kg) dose-dependently decreased the firing rate of substantia nigra reticulata neurons (Fig. 1B). Over the total dose range, substantia nigra reticulata activity decreased gradually to 70% of baseline activity, with very little variation between individual rats upon treatment. In comparison to saline treatment, risperidone significantly ($P < 0.01$) affected substantia nigra reticulata neuronal activity at a dose of 800–3200 μg/kg.

Clozapine (100–6400 μg/kg) also reduced the substantia nigra reticulata neuronal activity in a dose-dependent manner (Fig. 1C). At a dose of approximately 400 μg/kg the substantia nigra reticulata activity was maximally decreased to 70–75% of baseline activity, again with very little variation between individual experiments. Compared to saline treatment, clozapine significantly reduced substantia nigra reticulata activity at a dose of 800–6400 μg/kg with a significance level of $P < 0.01$ except for the effect at a dose of 1600 μg/kg which had a significance level of $P < 0.05$. In two experiments, the rat died after clozapine treatment, at $t = 20$ min and at $t = 35$ min after the experiment had been terminated, respectively. These experiments were included in the data in Fig. 1C.

Haloperidol (12.5–800 μg/kg) gradually induced a slight increase in substantia nigra reticulata activity (Fig. 1D). This increase, however, was not significantly different from the gradual increase observed after saline treatment. After one of the experiments was terminated, an additional chloralhydrate injection was administered to confirm the anaesthesia level as the cause of the gradual increase in activity of the substantia nigra reticulata neurons. After the injection, substantia nigra reticulata activity decreased back to baseline levels. One experiment was excluded from the data as the electrode placement was different (in the upper lateral part of the substantia nigra reticulata).

3.2. Effect of quipazine alone and in combination with risperidone on substantia nigra reticulata neuronal activity

After i.p. injection of quipazine (0.5 mg/kg) at $t = -15$ min, followed by saline i.v. injections starting from $t = 0$

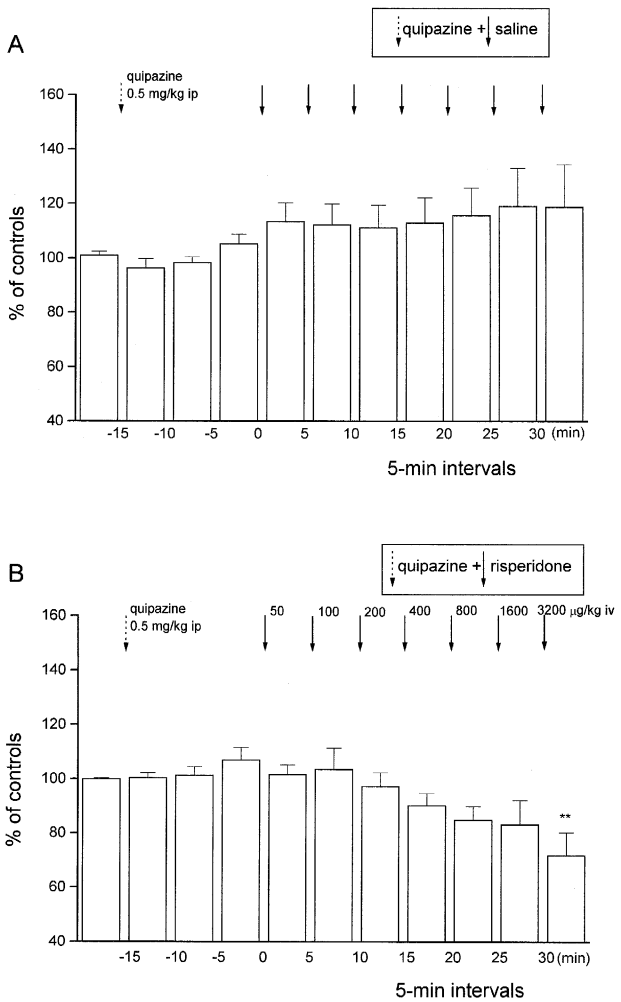

Fig. 2. Effects of (A) quipazine (0.5 mg/kg i.p.) followed by cumulative saline injections ($n = 4$) and (B) quipazine (0.5 mg/kg i.p.) followed by cumulative risperidone injections (50–3200 μg/kg i.v.; $n = 5$), respectively, on substantia nigra reticulata extracellular recordings. Data are expressed as percentages of baseline activity, determined from the average of successive 10-s bin counts obtained over a 5-min interval, shown in the first column. The other columns represent the average activity over 5-min intervals. All values are means ± S.E.M. ** $P < 0.01$ vs. saline.

to 30 min, using a protocol identical to that described earlier, substantia nigra reticulata activity was slightly increased during the entire experimental period (Fig. 2A). A similar gradual increase was observed without pretreatment with quipazine, probably as a consequence of the changing level of anaesthesia during the experiment. A dose of 0.5 mg/kg was used, based on studies of Olds and Yuwiler (1992). Higher doses of quipazine themselves had an effect on substantia nigra reticulata firing, preliminary data suggest that 1 and 5 mg/kg induced a dose-dependent decrease, while 10 mg/kg initially decreased but subsequently increased substantia nigra reticulata activity.

Cumulative doses of risperidone (50–3200 μg/kg) after pretreatment with quipazine (0.5 mg/kg i.p.) induced a gradual decrease in substantia nigra reticulata activity, starting at a dose of 200 μg/kg (Fig. 2B). The effect, however, was only significantly different ($P < 0.01$) from that of quipazine pretreatment followed by saline injections at a dose of 3200 μg/kg. Thus, these data suggest that, in combination with quipazine, a higher dose of risperidone is necessary to reduce substantia nigra reticulata activity compared to the effect of risperidone given alone, implying partial blockade of the response induced by risperidone by the 5-HT_2 receptor agonist.

4. Discussion

The present study shows that atypical and typical neuroleptics affect differentially the firing rate of the substantia nigra reticulata neurons. Risperidone and clozapine both decreased the unit activity of substantia nigra reticulata neurons, while haloperidol did not affect the activity of these neurons. The fact that risperidone and clozapine significantly affected substantia nigra reticulata activity in a very consistent manner, is remarkable. Mostly agents with affinity for GABA receptors have so far been found to be reliably effective after systemic administration, i.e., inhibition of substantia nigra reticulata firing after $GABA_A$ receptor stimulation and treatment with benzodiazepines (e.g., Waszczak et al., 1980; Mereu et al., 1987). The effects of risperidone and clozapine on substantia nigra reticulata firing rate have not been described before. Only the effect of clozapine on substantia nigra reticulata firing after pretreatment with amphetamine has been described (Kamata and Rebec, 1985). Upon systemic administration of dopaminergic drugs, inconsistent effects on substantia nigra reticulata firing have been reported, agonists eliciting variable responses or being excitatory. Dopamine receptor antagonists have merely been used in combination with agonist pretreatment (Rebec and Groves, 1975; Waszczak et al., 1984; Olds, 1988a,b), although Olds reported a modest decrease, statistically non-significant, in substantia nigra reticulata activity after a single dose of haloperidol in awake animals.

Thus, this is the first study in which dose ranges of risperidone, clozapine and haloperidol were studied for effects on substantia nigra reticulata activity as a reflection of the output activity of the basal ganglia. The present experimental setup could potentially serve as a model for classification of typical vs. atypical drugs, as is the case for the measurement of dopaminergic activity of the striatal vs. mesolimbic system (White and Wang, 1983).

4.1. The role of 5-HT_2 receptors

The inhibitory effect of risperidone upon the firing rate of substantia nigra reticulata neurons could be partially blocked by pretreatment with the 5-$HT_{2a/c}$ receptor agonist, quipazine. A 4-fold higher dose of risperidone was needed to induce a significant decrease in substantia nigra reticulata activity after pretreatment with quipazine. Since risperidone is a 5-HT_{2a} rather than a 5-HT_{2c} receptor antagonist (Meltzer, 1995) the effect can be ascribed to 5-HT_{2a} receptors. Limitations in duration of the effect of quipazine, but also the involvement of other than 5-HT_2 receptors may underlie the fact that the blockade was partial and not absolute. Although risperidone is a potent 5-HT_2 receptor antagonist, it also has considerable affinity for dopamine D_2, D_3 and D_4 receptors, next to α_1- and α_2-adrenoceptors, histamine H_1 receptors and 5-HT_6 and 5-HT_7 receptors (Leysen et al., 1992). The involvement of these receptors in the effect of risperidone on the substantia nigra reticulata output neurons has not been studied yet.

Thus, the 5-HT-component of the risperidone effect is, at least partly, responsible for the difference in response of substantia nigra reticulata neurons in comparison to the response to haloperidol. 5-HT_2 receptors are widely distributed in the brain, with highest densities in the cortex, less in, e.g., the striatum and nucleus accumbens and low concentrations in the ventral tegmental area, substantia nigra compacta and substantia nigra reticulata (Schotte et al., 1983; Pazos et al., 1985, 1987). Therefore, it cannot be excluded that the 5-HT_2 effect of risperidone on substantia nigra reticulata firing rate was mediated locally, within the substantia nigra reticulata. Studies regarding local effects of serotonin on substantia nigra reticulata neurons have yielded conflicting data, pointing to both an inhibitory and an excitatory role for serotonin locally in the substantia nigra reticulata (Dray et al., 1976; Fibiger and Miller, 1977; Oberlander et al., 1981; Rick et al., 1995). A source of pharmacological divergence may be the presence of serotonin receptor subtypes other than to 5-HT_2, i.e., 5-HT_1 and 5-HT_4 in the substantia nigra reticulata (e.g., Pazos et al., 1985; Grossman et al., 1993). Given the fact that there is a lack of data on in vivo receptor binding in the substantia nigra reticulata upon neuroleptic treatment, it remains unsolved whether the substantia nigra reticulata is the site for mediation of the atypical neuroleptic effect on substantia nigra reticulata activity. On the other hand, the cortex is a site which is most likely to be involved, as PET studies showed that risperidone induces a marked occu-

pancy of cortical 5-HT_2 receptors (approx. 60% receptor occupancy 4–7 h after oral administration of a single dose of 1 mg) in humans (Nyberg et al., 1993). Various pharmacological studies also indicate that risperidone and/or clozapine, but not haloperidol, interact with cortical 5-HT_2 receptors (e.g., Ashby and Wang, 1990; Gellman and Aghajanian, 1994). Additional studies, using local application of selective drugs, will broaden our insight into the brain areas potentially involved in mediating the effect on substantia nigra reticulata neurons.

4.2. Methodological considerations

The characteristics of the electrode microwire imply that the larger projection neurons rather than the interneurons present in the substantia nigra reticulata will be recorded (Olds, 1988b). The substantia nigra reticulata neurons recorded were not identified regarding their target structures. Given the fact that neurons were recorded from a broad medial area of the substantia nigra reticulata, it can be assumed that nigro-thalamic, nigrotectal, and nigrocollicular neurons might all have been recorded, based on the lamellar organisation of the substantia nigra reticulata in the rat (Deniau and Chevalier, 1992).

The 5-HT receptor agonist, quipazine, is described as a potent 5-HT_2 receptor agent, but also possesses affinity for the 5-HT_3 receptor (e.g., Robertson et al., 1992). The partial blockade of the inhibitory effect of risperidone on substantia nigra reticulata firing rate, however, can be assumed to be mediated via 5-HT_2 receptors, based on the lack of affinity of risperidone for 5-HT_3 receptors (Leysen et al., 1992).

The effect of clozapine on substantia nigra reticulata firing rate should be considered with some reserve, since the application of clozapine was occasionally lethal. We cannot rule out the possibility that part of the decrease in substantia nigra reticulata activity may have resulted from a worsening of the physiological condition of the rat as an aspecific consequence of clozapine treatment.

Although one can argue in favour of performing electrophysiological experiments in awake animals, pharmacological effects of various treatments can be conveniently differentiated using the anaesthetized preparation. Especially the stability of the firing rate of the substantia nigra reticulata neurons under the latter conditions enables small changes to be reliably monitored. The characteristics of the response, however, should be looked at with caution, since, for instance, the effects of dopamine on the electrical activity of striatal cells largely depend on the experimental conditions (Warenycia and McKenzie, 1984). A state-dependent effect also applies for neuroleptic drugs, as these agents are reported to accelerate striatal activity in the stereotaxic preparation (Rebec et al., 1979, 1980), but effectively inhibit amphetamine-induced excitation in awake animals (Haracz et al., 1993). On the other hand, Olds (1988a,b) showed identical responses of substantia nigra reticulata neurons to systemic administration of amphetamine/apomorphine in awake rats and in rats treated with a low dose of urethane.

The experimental approach used in this study reflects the activity of the basal ganglia output in the substantia nigra reticulata upon neuroleptic treatment. The internal segment of the globus pallidus, as the second major output structure of the basal ganglia, may well respond in a different way. Thus, the output of the basal ganglia as was studied here is biased towards that part of the information that is relayed via the substantia nigra reticulata to brain structures outside the basal ganglia.

4.3. Basal ganglia circuitry

The differential effects of atypical and typical neuroleptics on the mesolimbic vs. the striatal dopaminergic systems cannot hold for postsynaptic structures. Evidence from various disciplines points to a loss of limbic and striatal segregation at the postsynaptic level. Anatomical studies provide evidence for non-reciprocal synaptic contacts, allowing the ventral limbic-related striatum to communicate with the sensorimotor-related striatum through their connections with the substantia nigra (Nauta and Domesick, 1984; Lynd-Balta and Haber, 1994). In agreement with the anatomical data is the finding that a pharmacological challenge after lesioning of the nucleus accumbens affects substantia nigra reticulata rather than ventral tegmental area non-dopaminergic neurons (Olds, 1988a). Neuroleptics do not confine their activity to limbic regions, as is illustrated by the fact that neuroleptics have identical potencies in limbic and putamen regions in vivo (Seeman and Ulpian, 1983; Wilmot and Szczepanik, 1989). In line with this observation, results of studies in awake rats show that both haloperidol and clozapine block the amphetamine- or phencyclidine-induced activation of striatal neurons (Haracz et al., 1993; Wang and Rebec, 1993; White et al., 1995). On the other hand, immediate-early gene studies did show differences in response to typical and atypical neuroleptics in structures postsynaptic to the mesocortical and mesostriatal systems (e.g., Nguyen et al., 1992; Robertson and Fibiger, 1992; Fink-Jensen and Kristensen, 1994).

Thus, to consider certain activities at the postsynaptic level as reflecting extrapyramidal side-effects or antipsychotic activity after neuroleptic treatment should be done cautiously. Our data providing differential effects of atypical and typical neuroleptics on the firing rate of substantia nigra reticulata neurons may well reflect an integrated activity of limbic and motor parts of the basal ganglia after neuroleptic treatment, that is processed via the substantia nigra reticulata neurons on brain structures outside the basal ganglia. On the other hand, the fact that risperidone and clozapine, but not haloperidol, reduced the firing rate of the substantia nigra reticulata neurons may suggest (1) that changes in substantia nigra reticulata activity are not

associated with the antipsychotic activity of neuroleptics, or (2) that a decrease in substantia nigra reticulata output may be a mechanism by which clozapine and risperidone counter extrapyramidal side-effects. The latter relationship is of course not causally proven, and therefore remains speculative, but given the large body of evidence regarding the involvement of substantia nigra reticulata neurons in a neuronal circuitry responsible for mediating motor responses (for review, see DeLong, 1990), this hypothesis is attractive and should be further investigated. If valid, it would suggest that blockade of 5-HT_2 receptors determines the activity level of the substantia nigra reticulata neurons as such that dopamine blockade does not per se result in extrapyramidal side-effects. However, considering the dose range of risperidone that affected substantia nigra reticulata activity in relation to doses effective to induce catalepsy as a measure for extrapyramidal side-effects (Leysen et al., 1993), there is very little difference in dose, assuming the two experimental conditions may be compared. Even if the present hypothesis does apply for risperidone, it is not a general phenomenon to explain the lack of extrapyramidal side-effects after atypical neuroleptic treatment, as for instance, clozapine does not induce the $>80\%$ dopamine D_2 receptor occupancy necessary for the induction of extrapyramidal side-effects (Farde et al., 1992) in the clinic (Farde and Nordstrom, 1992), while other atypical neuroleptics lack 5-HT_2 affinity (e.g., remoxipride, sulpiride). Thus, at the moment it is unclear how the results in this study should be interpreted in terms of antipsychotic and/or extrapyramidal side-effects activity. Studies on the effects of long-term treatment with the various neuroleptics on substantia nigra reticulata and ventral tegmental area non-dopaminergic neuronal activity, preferably in awake rats, should broaden our insight into the phenomena underlying the mechanisms of action of these therapeutic agents.

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