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Research report

Behavioural effects of chronic haloperidol and risperidone treatment in rats

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Abstract

The therapeutic properties of typical antipsychotic drugs (APDs) such as haloperidol in schizophrenia treatment are mainly associated with their ability to block dopamine D2 receptors. This blockade is accompanied by side effects such as extrapyramidal symptoms (EPS). Atypical APDs such as risperidone have superior therapeutic efficacy possibly due to their activity at multiple receptors (in particular 5-HT2A receptors). Although the risk of EPS is significantly lower in atypical than in typical APDs, it is not negligible. To investigate and compare the behavioural profile and EPS-asssociated side effects of haloperidol and risperidone APD treatment we applied a multi-tiered, comprehensive behavioural phenotyping approach. Sprague-Dawley rats were treated chronically (28 days) with supratherapeutic EPS-inducing doses of haloperidol and risperidone using osmotic minipumps. Domains such as motor activity, exploration, memory, and anxiety were analysed together with EPS assessment ("early onset" vacuous chewing movements and catalepsy). Both APDs produced diminished motor activity and exploration, impaired working memory performances, and increased anxiety levels. These effects were more pronounced in haloperidol-treated animals. Chronic APD treatment also caused a time-course dependent elevation of EPS-like symptoms. Risperidone-treated animals showed a catalepsy-like phenotype, which differed to that of haloperidol-treated rats, indicating that processes other than the anticipated dopaminergic mechanisms are underlying this phenomenon. These EPS-related phenotypes are consistent with reported EPS-inducing D2 receptor occupancies of around 80%. Differences in the behavioural profile of haloperidol and risperidone, which were revealed by a comprehensive phenotyping strategy, are likely due to the unique receptor activation profiles of these APDs.

Keywords: Sprague-Dawley rat; Antipsychotic drug; Haloperidol; Risperidone; Osmotic pump; Motor activity; Exploration; Learning and memory; Extrapyramidal symptoms; Anxiety

1. Introduction

Current knowledge on the neurochemistry underlying the pathogenesis of schizophrenia implies alterations in neurotransmitter systems such as dopamine (D), glutamate, and serotonin (5-HT) [1]. These systemic alterations possibly trigger the classical features of schizophrenia, which include positive (e.g. hallucinations) and negative (e.g. amotivation) symptoms and cognitive deficits (e.g. in attention). Although negative symptoms appear to be less disturbing than positive, they are debilitating and more difficult to reverse.

Antipsychotic agents have been the mainstay in the management of schizophrenia for a number of years. Conventional or typical antipsychotic drugs (APDs), such as haloperidol, reverse positive symptoms but are not particularly effective against negative symptoms or cognitive deficits. Since positive symptoms of schizophrenia are suggested to arise from hyperdopaminergic activity, especially from the over-activation of dopamine D2 receptors, typical APDs release their therapeutic properties mainly by blocking these receptors (blockade of up to 80% in the basal ganglia is required). This D2 receptor blockade is accompanied by disturbing and incapacitating side effects, so-called extrapyramidal symptoms (EPS), which include tremor, rigidity, akinesia or bradykinesia [2]. Haloperidol exhibits low activity at 5-HT2A, D1, and α_1 and α_2 adrenergic receptors, and only minimal affinity to 5-HT1A and histamine (H1) receptors [3]. In contrast, more recently developed so-called atypical APDs, such as risperidone, have superior therapeutic efficacy in the

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treatment of not only positive but also negative symptoms of schizophrenia as well as cognitive impairments, although the latter effect is limited [4]. The improved efficacy of risperidone is due to its activity at multiple receptors, in particular its primary actions on the 5-HT2A receptor (up to 90% blockade) and relatively lower affinity to D2 receptors compared to haloperidol (less than 80% blockade) in the mesocorticolimbic neuronal circuit [5]. Risperidone is also characterised by low affinities to α_1 and α_2 adrenergic receptors and H1 receptors, and further by minimal activity at other serotonergic and dopaminergic receptors [6,3]. Importantly, risperidone's differential receptor affinity and its serotonergic modulation of the D pathway [6] results in a much lower risk of EPS and tardive dyskinesia. However, the drug loses its 'atypicality' or resistance to EPS when a high drug dosage is used, resulting in blockade of around 80% of dopamine D2 receptors [7,8]). Other common side effects produced by atypical APD treatment can be weight gain, hyperglycemia, and *diabetes mellitus*.

The investigation and comparison of EPS and other potential side effects of APD treatment is highly important for the clinical population as more than 70% of schizophrenia patients treated with (a)typical APDs discontinue their medication within 18 months because of the side effects accompanying drug treatment [9]. Importantly, although the risk of EPS caused by risperidone in clinical practice is significantly smaller than by haloperidol, it is not negligible [10]. Obviously, there is a need to elucidate the behavioural profile of these drugs and the underlying molecular mechanisms in more detail to develop better and more specific drug treatments for schizophrenia [11]. Various animal models are available for a behavioural characterisation of typical and atypical APDs. Unfortunately, most animal studies only perform a limited phenotyping strategy, focusing only on EPS, and use a wide variety of different drug treatment designs (dosage, mode of administration, duration of treatment period) (e.g. [12–15]). Therefore, it would be challenging to cross-correlate behavioural findings of these animal studies. Besides, the effects of neuroleptic treatment on memory [16,17] and anxiety [18–24] are discussed controversially.

In this study, we aimed to produce a well-characterised and -standardised chronic (28 days) animal model of supratherapeutic and therefore EPS-inducing risperidone and haloperidol treatment using osmotic minipumps. As risperidone achieves D2 receptor occupancies less readily than haloperidol, it was administered in proportionately higher doses (2.13 mg/kg/day) relative to haloperidol (0.4 mg/kg/day) to exceed the 80% D2 occupancy [25,26,8] thereby producing EPS [27]. The choice of the mode of drug administration was justified by the reported half-life of APDs in rodents that is 4–6 times shorter than in humans. Therefore, continuous drug administration was needed to achieve occupancies comparable to clinical use in humans [26]. Furthermore, minipumps are highly reliable and less disturbing than other modes of drug administration. Importantly, animal studies can model certain behavioural aspects of human EPS. "Early onset" vacuous chewing movements (VCMs) induced by subchronic APD treatment in rats are proposed to model dystonic movements and tremor observed in human EPS and in Parkinson's disease, while rat catalepsy models APD-induced akinesia and rigidity [14].

Thus, we applied a unique multi-tiered, comprehensive behavioural phenotyping approach to characterise and clarify the effects of chronic haloperidol and risperidone treatment on EPS and associated behavioural side effects in the Sprague-Dawley (SD) rats. We focused on various behavioural domains such as motor activity, exploration, anxiety, and learning and memory. We hypothesized that chronic neuroleptic treatment at supratherapeutic doses not only leads to EPS-like symptoms but may also result in gross changes in other behavioural domains such as anxiety and learning. However, due to differences in receptor affinities, we expect haloperidol and risperidone to demonstrate specific behavioural profiles. Such a complex phenotyping strategy is a very useful tool in schizophrenia research and the evaluated behavioural profile provides the research community with a well-characterised and -established animal model that can be taken further by investigators to study the molecular mechanisms behind these drug effects and in particular the mechanisms underlying EPS. These studies will propel our knowledge on APDs mode of action and may help to reduce side effects and increase drug efficacies. Furthermore, controversies about the effects of haloperidol and risperidone on memory and anxiety can be clarified. Thus, the comprehensive and standardised behavioural profiling of haloperidol and risperidone in a chronic SD rat model provides the first critical step for the development of better and more specific treatments for schizophrenia.

2. Materials and methods

2.1. Animals

Forty-eight age-matched adult, male Sprague-Dawley rats obtained from the animal facility of the University of Adelaide, Laboratory Animal Services, Adelaide, Australia [9–10 weeks at arrival, 357 ± 75 g body weight (BW)] were kept under standard laboratory conditions with a 12:12 h light:dark schedule (light phase: white light with illumination of 70 lx – dark phase: red light with illumination of <2 lx). After transfer, rats were allowed to habituate to the new holding facility for 7 days. Test animals were allocated to four different experimental groups ($n = 12$ rats/group): (1) "control": no treatment; (2) "vehicle": osmotic pump implants loaded with vehicle solution; (3) "haloperidol": osmotic pump implants loaded with haloperidol; (4) "risperidone": osmotic pump implants loaded with risperidone. Animals were kept group-housed (three rats of similar treatment per cage). For habituation all animals were transported to the test room 1 h prior to behavioural testing, which was performed during the light phase of the light cycle (for age of rats and test order see Table 1). All research and animal care procedures were approved by the "Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee" and were in agreement with the "*Australian Code of Practice for the Care and Use of Animals for Scientific Purpose*".

2.2. Drugs and implantation of osmotic pumps

Supratherapeutic doses of haloperidol (0.4 mg/kg/day) and risperidone (2.13 mg/kg/day) were continuously administered for 4 weeks using osmotic minipumps (Model 2ML4: Alzet, Palo Alto, CA, USA). Drugs (amounts calculated for 28 days continuous infusion) were dissolved in 20% acetic acid solution, then diluted with sterile water to a final volume of 2 ml, and buffered to pH 5.0 ± 0.3 with 10 M sodium hydroxide. Osmotic minipumps were loaded with: (i) vehicle solution (pH-adjusted acetic acid solution), (ii) haloperidol (averaged 0.4 mg/kg/day BW), or (iii) risperidone (averaged 2.13 mg/kg/day BW) [28]. Using halothane anaesthesia, minipumps were implanted subcutaneously