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Acute antipsychotic treatments induce distinct c-Fos expression patterns in appetite-related neuronal structures of the rat brain

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ABSTRACT

A number of atypical antipsychotic drugs are known to perturb appetite regulation causing greater hyperphagia in humans and rodents than earlier generation typical agents. However, the neuronal structures that underlie hyperphagic effects are poorly understood. Arcuate nucleus (ArcN), paraventricular hypothalamic nucleus (PVN), paraventricular thalamic nucleus (PVA) and nucleus incertus (NI) have been implicated in appetite regulation. The NI is the principal source of the relaxin-3 (RLN3) peptide, which is reported to have orexigenic effects. Moreover, ArcN, PVN, and PVA receive RLN3 immunoreactive fibers from the NI and express relaxin family peptide type 3 (RXFP3) receptor. The present study was designed to evaluate the acute effects of clozapine (atypical), chlorpromazine (typical) and fluphenazine (typical) on c-Fos expression (a marker of neuronal response) in these appetite-related centers of the rat brain. The numbers of c-Fos expressing neurons in these structures were counted in immunofluorescence stained brain sections. Acute treatment with clozapine, chlorpromazine and fluphenazine differentially influenced c-Fos expression in these brain structures. This study is also the first demonstration that antipsychotics influence the NI. The patterns of the effects of these antipsychotics are related to their reported hyperphagic properties.

1. Introduction

Weight gain and related metabolic adverse effects are prominent side effects associated with antipsychotic drug treatment. One mechanism contributing to weight gain, on which a number of the newer generation atypical antipsychotic drugs in particular have more pronounced effects, is hyperphagia. Clinical studies have shown that clozapine, an atypical antipsychotic, causes enhanced food intake (Theisen et al., 2003; Gebhardt et al., 2007) and such effects contribute to the weight gain associated with chronic treatment (Leadbetter et al., 1992; Hummer et al., 1995;

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Brömel et al., 1998). There have been relatively fewer reports on typical antipsychotic-induced enhanced food intake in humans (Silverstone et al., 1988; Ganguli, 1999) indicating that development of atypical antipsychotics has in general failed to manage the weight gain problems associated with the earlier generation and in some cases has in fact worsened it. The preclinical evaluation of antipsychotic-induced hyperphagia began several decades ago. Observations on systemic chlorpromazine treatment (Robinson et al., 1975) and both central and systemic clozapine treatment (Antelman et al., 1977) showed significant increases in food intake. Similar to the clinical studies, further studies in rodents have shown relatively lower (or even absence) of hyperphagia in response to typical antipsychotic treatment (Baptista et al., 1987; Allison et al., 1999). A large body of data from rodent feeding behavior studies has demonstrated the hyperphagic effect of many atypical antipsychotics, especially clozapine. To mention a few, in male hooded Lister rats, clozapine treatment increased the meal size in the first two hours after drug administration (Lee and Clifton, 2002) and when observing the microstructure of the ingestive behavior in these rats, clozapine was found to augment intake in the short fat drinking paradigm (Hartfield et al., 2003). In Sprague-Dawley rats, clozapine impaired appetite regulation systems by increasing plasma ghrelin within 60 min following administration (Murashita et al., 2007).

Mapping of the expression of c-Fos, an immediate early gene, in response to drug administration has been proven to provide information on brain region specificity of drug effects. In particular, studies have utilized this technique to map the brain regions that are influenced by antipsychotic drugs (Fink-Jensen and Kristensen, 1994; Deutch and Duman, 1996; Cohen et al., 2003; Sumner et al., 2004). Moreover, c-Fos expression profiles of atypical and typical antipsychotics in a variety of neuronal structures are markedly different and this method has been used to assess differences in the mechanisms of action between the generations of antipsychotics (Robertson and Fibiger, 1992; Deutch et al., 1992; Robertson et al., 1994; Fadel et al., 2002; Oka et al., 2004).

The arcuate nucleus (ArcN) is thought to be the hypothalamic structure most involved in the control of food intake, and predominant aspects of its control comes from the neuropeptidergic systems involved and via its projections to other hypothalamic structures (Johnstone et al., 2006; Schwartz et al., 2000). Likewise, the hypothalamic paraventricular nucleus (PVN) is richly supplied by the axons of the neuropeptide Y/agouti-related peptide (NPY/AGRP) and pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript neurons (POMC/CART) originating from the ArcN, forming a site for second-order neuronal signaling pathways (Elmquist et al., 1998, 1999). The paraventricular thalamic nucleus (PVA), known to have altered c-Fos expression in response to treatment with certain antipsychotics (Cohen et al., 1998), demonstrates meal-time synchronized circadian rhythms of glucose utilization, c-Fos expression and clock gene expression (Mendoza et al., 2005; Angeles-Castellanos et al., 2007) and recent reports have shown its role in food motivation (Choi et al., 2010, 2012) inspiring the need to further study the response of the PVA to antipsychotic treatment.

Relaxin-3 (RLN3) has been implicated in feeding behavior. The nucleus incertus (NI) is the chief source of RLN3 in the mammalian brain. RLN3 immunoreactive fibers have been detected to innervated ArcN, PVN and PVA (Bathgate et al., 2003; Tanaka et al., 2005; McGowan et al., 2007; Ma et al., 2007; Ryan et al., 2011). However, tracing studies did not identify connections from NI to ArcN and PVN raising the possibility that the RLN3 immunoreactive fibers seen in these nuclei originate from another source (Goto et al., 2001; Olucha-Bordonau et al., 2003; Ryan et al., 2011). In addition, RXFP3 receptor has been identified in ArcN, PVN and PVA (Sutton et al., 2004; McGowan et al., 2007; Ma et al., 2007; Ryan et al., 2011). Orexigenic effects of RLN3 have been observed in studies in which the peptide was administered via intracerebroventricular (ICV) or unilateral intra-paraventricular (iPVN) injections (McGowan et al., 2005, 2006), as well as on injection into the ArcN and anterior preoptic area (APOA) (McGowan et al., 2007). All these reports suggest that RLN3 is a potent orexigen, and that its actions at sites such as the ArcN, PVN and PVA may be important in controlling feeding behavior.

We sought to compare the c-Fos induction effects of clozapine, an atypical antipsychotic drug with a high tendency to cause hyperphagia, with chlorpromazine and fluphenazine, two typical antipsychotics that have relatively moderate and low hyperphagic potential, respectively (Gray et al., 1993; Allison et al., 1999; Kaur and Kulkarni, 2002; Baptista et al., 2002). The brain regions of interest, as described above, included the ArcN and PVN of the hypothalamus, the PVA and the NI.

2. Results

Rats treated with saline, clozapine (15 and 30 mg/kg), chlorpromazine (10 and 100 mg/kg) or fluphenazine (3 and 10 mg/kg) were sacrificed 2 h after drug administration. c-Fos immunoreactivity was investigated in ArcN, PVN, PVA and NI (Fig. 1). c-Fos expression was observed in the nucleus (Fig. 2). For the NI, immunostaining of corticotrophin releasing factor type 1 and 2 ($CRF_{1/2}$) receptors was used as a marker to define the nucleus (Tanaka et al., 2005) and nuclear c-Fos expression co-localized with $CRF_{1/2}$ receptor immunoreactivity in the cytoplasm (Fig. 2). The c-Fos positive cells were counted. One-way analysis of variance (ANOVA) showed that all the treatments significantly influenced c-Fos expression in ArcN [$F6, 41=36.568$, $P<0.001$], PVN [$F6, 41=46.316$, $P<0.001$], PVA [$F6, 41=36.647$, $P<0.001$] and NI [$F6, 41=48.822$, $P<0.001$].

2.1. c-Fos immunoreactivity in the ArcN

Post hoc Tukey's HSD tests showed that clozapine (15 and 30 mg/kg, s.c.) significantly ($P<0.001$) increased the number of c-Fos positive cells in the ArcN when compared to vehicle (Fig. 3). Likewise, chlorpromazine treatments (10 and 100 mg/kg, s.c.), significantly ($P<0.05$ and $P<0.01$, respectively) increased the number of cells expressing c-Fos when compared to the vehicle group. On the other hand, fluphenazine (3 and 10 mg/kg, s.c.) did not show any significant increase