Haloperidol reduces IgG immunoreactivity in the rat brain

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Abstract
Immunohistochemical staining with non-specific IgG reliably labels a subset of neurons in both rat and human brain, overlapping the distribution of cortico-limbic dopamine D2 receptors (D2R). We used haloperidol to up-regulate rat D2R to observe any associated changes in IgG immunostaining. Rats were treated with haloperidol or vehicle for 30 d. Some rats were assessed for D2R up-regulation with apomorphine. The remaining rats were processed immunohistochemically and IgG-stained cells counted and statistically analysed in three cortico-limbic areas. Other brain regions were surveyed qualitatively. Positive (anti-glial fibrillary acidic protein or anti-tyrosine hydroxylase staining) and negative (antisera omitted) controls were performed on adjacent sections. Haloperidol dramatically reduced the IgG staining in areas quantified with all surveyed regions significantly decreased. Positive control staining was robust, ruling out generalized immunoreactivity reduction. These observations raise the possibility of an immunological effect of haloperidol in the brain. The identification and function of these IgG-labelled sites may have useful implications for psychosis.

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Introduction
Current evidence points toward neurodevelopmental (Lobato et al., 2001) and immunological (Muller et al., 2000) variables in schizophrenia, although brain mechanisms mediating these influences are unclear. Neurodegeneration and neural injury observed in other developmental or immunological disorders are absent in schizophrenia (Arnold, 2001), although reduced regional brain volume has been observed (DeLisi, 1999; Thompson et al., 2001).

Immunohistochemical staining of a subset of neurons by non-specific, polyclonal immunoglobulin-G (IgG) has been typically viewed as a methodological artifact (Schmidt-Kastner et al., 1993), but comparison of regional distributions suggest otherwise. Immunohistochemical staining of human and rat brain with non-specific IgG (from all species tested as described below) overlaps the regional distribution of dopamine D2 receptors (D2R) (Goldsmith et al., 1991) in circuits implicated in schizophrenia (DeLisi, 1999). There is high regional concordance between D2R and IgG staining in cortico-limbic circuits including frontal cortex, hippocampus, and nucleus accumbens. However a complementary pattern is observed in parahippocampal cortices. There are high densities of D2R in perirhinal cortex and no detectable D2R in a target of perirhinal afferents, the entorhinal cortex. IgG immunostaining is the opposite in parahippocampal regions, with dense staining in the entorhinal cortex, but none observed in the perirhinal cortex. Subcortical structures show no obvious relationship between D2R and IgG staining: no IgG immunostaining in substantia nigra (second highest concentration of D2R), but dense IgG staining of the red nucleus (no reported D2R). There are also intraneuronal discordances. IgG neuron staining is densest over cell nuclei but less dense over somata; the apical dendrites of deep cortical pyramidal cells are also occasionally stained (Goldsmith et al., 1991). In comparison D2R have been localized primarily to somata, dendrites and axon terminals (Delle Donne et al., 1996, 1997).

The cortico-limbic circuits stained with IgG are implicated in schizophrenia (DiLisi, 1999). Although there is a well-established association between D2R up-regulation and symptom relief from neuroleptics, the mechanism of the clinical efficacy remains unknown.
In addition, there appears to be immunological involvement in schizophrenia (Muller et al., 2000). If these IgG-stained sites are pertinent to schizophrenia, then they would be expected to be sensitive to neuroleptics. In order to test for neuroleptic sensitivity of the IgG-stained sites, rats were treated with the antipsychotic drug haloperidol (Hal), which increases D2R concentrations in rats (Creese et al., 1977), to test for any associated change in IgG immunostaining. There have been no prior reports of changes in brain immunoreactivity in response to neuroleptics.

Methods

Experiments were performed in accordance with accepted guidelines (DHEW, 1980). Rats were injected intraperitoneally daily for 30 d with either 0.75 mg/kg d Hal (n = 17) or an equivalent volume of vehicle as controls (Ctl, n = 13). (These data represent the experiment and a replication, and are presented together since there were no significant differences across replication.) Prior to behavioural assessment, tissue processing and neuron counting, experimental and Ctl animals were coded. Thus all observations were made blind to the treatment given to a particular animal. Ninety-six hours after the last injection, 6 treated and 6 Ctl rats were injected with the dopamine agonist, apomorphine. Resulting stereotypies have been established as a sensitive behavioural assay for the density of D2R in rats (Creese et al., 1977). Therefore, behavioural stereotypies were quantified as an indication of the D2R response to Hal or vehicle treatment, and were statistically analysed using Student’s t test. The remaining rats (n = 18) were processed for brain immunohistochemistry as described previously (Goldsmith and Joyce, 1994). Raw, primary antiserum (source of non-specific IgG), secondary antiserum (peroxidase-labelled goat anti-IgG), anti-glial fibrillary acidic protein (anti-GFAP), and anti-tyrosine hydroxylase (anti-TH) antibodies were obtained from either Jackson ImmunoResearch Laboratories (West Grove, PA, USA) or Sigma Chemical Co. (St. Louis, MO, USA). It should be noted that initial studies using raw anti-sera or purified IgG from humans, goats or rabbits as the primary antisera, all stained the same subset of neurons in both rat and human brain. Human IgG was used for these experiments. Specific antibodies against GFAP and TH were used as positive controls; to ensure that any observed changes in IgG immunoreactivity were not due to a general, or wholesale change in tissue immunoreactivity under present laboratory conditions. Negative control sections were incubated in media that contained either no primary or no secondary antisera.

In each rat, stained cells were counted in one ×10 microscope field for 3 sections from somatosensory cortex area 9, CA1-subiculum of the septal hippocampus, and nucleus accumbens. Counts were taken at approximately the same rostral-caudal level for each region across rats. Means per region per rat were entered into a MANOVA to test for treatment effects. Simple analysis of variance (ANOVA) of each region was used to follow up significant MANOVA results. Brain regions that were not quantified were surveyed for qualitative changes in IgG immunostaining in response to Hal.

Results

Apomorphine challenge caused significantly more behavioral stereotypies in Hal vs. Ctl rats (t = 4.836, p = 0.01), confirming D2R up-regulation by Hal treatment (Creese et al., 1977). However, this presumptive increase in D2R was not accompanied by increased neuronal IgG staining. Instead as shown in Figure 1, chronic Hal significantly reduced IgG staining of neurons in all three quantified regions (MANOVA: F = 192.329, d.f. = 3,14, p = 0.0001). Simple ANOVAs performed on each quantified area confirmed highly significant (p < 0.0001), Hal-associated reductions in IgG staining. In contrast, immunostaining for positive controls (GFAP and TH) was robust in both treatment groups, indicating that there was no Hal-related, generalized reduction in immunoreactivity. Negative immunohistochemical controls resulted in no neuronal staining.

Qualitative surveys of other brain regions also revealed consistent decreases in IgG immunostaining in Hal rats. No regions surveyed showed any increase in IgG staining. IgG staining in the entorhinal cortex, robust in untreated rats, was virtually abolished after chronic Hal treatment. Chronic Hal treatment also markedly reduced IgG staining in the red nucleus, although staining was not completely eliminated there.

Discussion

This study identified an IgG-labelled site in the rat brain with a discrete anatomical distribution, that is drastically reduced by chronic Hal. The neuroleptic-induced reduction in IgG-labelled sites within this subset of rat cortico-limbic neurons raises questions of their potential relevance for psychotic disorders.

Hal affects receptors other than D2R hence the drastic reduction in IgG immunoreactivity could be
caused by mechanisms unrelated to D2R up-regulation. For example, Hal binds to \( \sigma \) receptors (Itzhak and Stein, 1990), and chronic treatment with Hal reduces \( \sigma \)-receptor concentrations in the rat brain (Inoue et al., 2000). The possibility that the IgG staining is recognizing \( \sigma \) receptors was considered, but there are sufficient disparities between the published findings on \( \sigma \) receptors, and the present observations of IgG staining, to make it unlikely. Although there is significant overlap for a number of brain regions with both \( \sigma \) receptors (Bouchard and Quirion, 1997) and the IgG staining observed here, including the red nucleus, isocortex and hippocampus, laminar and sub-field distributions are not the same. For example, \( \sigma_1 \) receptors (Bouchard and Quirion, 1997) are found in the dentate gyrus compared to IgG staining found only in the CA1-subiculum subfields of the hippocampus. \( \sigma_1 \) and \( \sigma_2 \) receptors were found in the middle layers of isocortex, compared to the IgG staining which labelled deep cortical pyramidal cells in isocortex. Moreover, these same researchers (Bouchard and Quirion, 1997) found \( \sigma_3 \) receptors in the substantia nigra pars reticulata, whereas there was no IgG staining of any part of the substantia nigra. Finally, the effects of chronic Hal on reducing regional \( \sigma \)-receptor densities in the rat brain, although significant, show only moderate effects,

Figure 1. Hal-reduced IgG immunostaining (B, D, F) in three regions of the rat brain compared to staining within these same regions in vehicle-treated rats (A, C, E). Statistical analysis (simple ANOVA) within each region indicated that the reduction in IgG staining following chronic Hal treatment was highly significant \( (p < 0.0001) \) within each of the three areas quantified.
with all regions retaining greater than 50% of control levels (Inoue et al., 2000), whereas chronic Hal essentially abolishes the IgG staining.

No other obvious candidates for the identity of this IgG-labelled site could be found in the literature, and it does not appear that the IgG staining is recognizing a sub-population of D2R given the disparities in anatomical distributions, most notably the lack of IgG staining in the substantia nigra and dorsal striatum, and the strong IgG staining in the red nucleus, where there are no reported D2R. Yet, downstream effects of D2R activity may be pertinent to the expression of these IgG-labelled sites. There are two reasons to consider this possibility: an anatomical observation in this study and; the body of published data on the relationships between central dopaminergic systems and the immune system. First, the effects of the Hal treatment on IgG staining in the entorhinal cortex raise the possibility of D2 modulation of these sites. Under control conditions, there is robust IgG staining in the entorhinal cortex, which has no measurable D2R in the rat (Goldsmith and Joyce, 1994). Yet, the Hal treatment abolished the entorhinal IgG staining, probably via its activity in the perirhinal cortex where there are significant levels of D2R in the rat (Goldsmith and Joyce, 1994), since the perirhinal cortex is a rich source of afferents to the entorhinal cortex.

Secondly, there is a growing body of clinical and experimental research revealing immunomodulation by the central dopaminergic system. In general, conditions which reduce activity of the nigrostriatal and/or mesolimbic dopaminergic pathways are associated with reduced immune function, including Parkinson’s disease (Marttila et al., 1985), lesions to cell body nuclei or target areas of these dopaminergic systems in rats (Devoino et al., 1997), or pharmacological antagonism of brain D2R including with Hal (Devoino et al., 1994). Conversely, treatment with dopamine agonists is associated with increased immune response (Devoino et al., 1994), and systemic immune challenge, for example following intraperitoneal injection of sheep red blood cells into rats, is associated with increased concentrations of dopamine in the mesolimbic and nigrostriatal pathways (Devoino et al., 1997). Research by Gomez et al. (1999) demonstrated that macrophage Fcγ receptor expression is modulated by systemic dopaminergic drugs. Specifically they found that pre-treatment with dopamine agonists enhanced, and antagonists reduced in-vitro clearance of IgG-sensitized red blood cells. This effect appeared to be mediated through altered expression of the macrophage Fcγ receptors, with D2 antagonists having a greater effect than D1 antagonists in reducing the expression of splenic macrophage Fcγ receptors (Gomez et al., 1999).

It would be interesting to test if the IgG staining in this study was via the Fc receptor portion of the antibodies, since Fc receptor expression is reduced in other tissues by Hal (Gomez et al., 1999). Yet all studies of Fc receptors in the brain find no significant expression in neurons (Bhatia et al., 1998), with all localization found either in microglia (e.g. for humans, Peress et al., 1993, and for rats Vedeler et al., 1994), or in the microvasculature and choroid plexus epithelium (Schlachetzki et al., 2002). Therefore, it seems unlikely that this discrete distribution of IgG staining is recognizing Fc receptors on neurons of the untreated, Ctrl rats. Alternatively, the IgG binding could be occurring via the Fc portion of the antibodies to another binding site, which is also regulated by the dopamine system. Such a binding site could be abnormally expressed in schizophrenia, or overly exposed to circulating IgG since there are some reports of increased presence of intrathecal IgG in schizophrenia (e.g. Muller and Ackenheil, 1995). Future experiments on the identity, function and regulation of this IgG-labelled site, may provide data pertinent to other observations including the presence of intrathecal IgG observed in schizophrenia (Muller and Ackenheil, 1995) and, the intrathecal synthesis of antibodies observed in animal models (Knopf et al., 1998), as well elucidating any other roles of this IgG-binding site in causing and/or ameliorating psychotic disorders.

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