A correlation of haloperidol-induced cognitive deficit with dysfunctional dopamine receptor activity in nonhuman primate

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Abstract: Haloperidol is an antipsychotic drug that acts through blockage of dopamine D2 receptors. Chronic administration of this antipsychotic drug in nonhuman primates induces a pronounced cognitive deficit. However, receptor subtypes that are responsible for this cognitive dysfunction remain unknown. Therefore, brains of chronic haloperidol-treated young and aged monkeys were used to analyze the intricate relation of receptor activity, cognitive dysfunction, and haloperidol-mediated actions in the production of harmful effects. Taking into account the significant cognitive loss observed after haloperidol treatment, it was predicted that changes in the cognitive status that correlate with the receptor activity in the prefrontal cortex and striatum, areas implicated in the processing of haloperidol-mediated effects in brain, should be common in both young and aged animals. Based on this concept, we observed that in the prefrontal cortex, dopamine D1 and D2 receptors showed changes in receptor levels that were common in both age groups. However, this relationship was absent in GABA, serotonin 5HT2 and muscarinic receptors. In contrast to the prefrontal cortex, in striatum, this change was restricted to the dopamine D2 receptors only. Therefore, from our results, it seems that apart from the downregulation of D1 receptor activity in the prefrontal cortex, an upregulation of D2 receptors could also contribute to the generation of the cognitive loss observed in haloperidol-treated monkeys. Additionally, reduced excitatory input due to hampered cortico-striatal D1 dopaminergic activity and stronger inhibition at the synapse of excitatory input site by upregulated striatal D2 receptor activity could promote the side effects associated with haloperidol.

Keywords: cognitive deficit, nonhuman primates, haloperidol, antipsychotic drug, receptor activity

Introduction

Schizophrenia is a disease that, in addition to cognitive dysfunction, is composed of negative1,2 and psychotic symptoms.3 Current knowledge on the neurochemistry underlying the pathogenesis of schizophrenia implies alterations in neurotransmitter systems such as dopamine, glutamate, and serotonin.4 Chronic antipsychotic drug administration is a crucial component in the current treatment of schizophrenia. Conventional or typical antipsychotic drugs, such as haloperidol, reverse psychotic 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 antipsychotic drugs release their therapeutic properties mainly by blocking these receptors. This D2 receptor blockade is accompanied by disturbing and incapacitating side effects, so-called extrapyramidal symptoms, including...
a parkinsonism-like syndrome and tardive dyskinesia. More than 70% of schizophrenia patients treated with typical antipsychotic drugs discontinue their medication within 18 months because of the side effects accompanying their drug treatment. Haloperidol is a potent D₂ antagonist that exhibits low activity at 5-HT2A, D₃, and adrenergic receptors, and has only minimal affinity to 5-HT1A and histamine (H₁) receptors. Animal studies have demonstrated that prolonged treatment with typical antipsychotics not only produces cognitive abnormality, but also induces purposeless chewing behavior and orofacial movements that are thought to result from potent D₂ blockade and a disruption of the balance between the D₁ and D₂ receptors.

The cortico-striatal pathway is involved in cognitive processing including the selection and execution of action. The striatum receives excitatory input from the cerebral cortex and thalamus and dopaminergic innervation from the midbrain. Dopaminergic activity modulates the flow of information through the striatum and the plasticity of cortico-striatal synapses. It is thought that dopaminergic activity in the cortico-striatal pathway plays an important role in not only haloperidol-mediated antipsychotic activity, but also undesirable side effects.

Schizophrenics have been shown to exhibit abnormal activities in the prefrontal cortex, but a blockade of up to 80% of D₂ receptors in the striatum is required for the antipsychotic effect to take place in these patients. However, it has been suggested that mechanisms that interfere with the dopaminergic, noradrenergic, and cholinergic activity in the prefrontal cortex might be responsible for both the beneficial and negative side effects related to haloperidol treatment in schizophrenics.

The prefrontal dysfunction has been shown to have the greatest effect on cognitive behavior. Findings of the induction of cognitive impairments after chronic haloperidol administration to young adult monkeys, and the reversal of this loss by activation of dopamine D₂ receptors, suggest a direct implication of dopaminergic neurotransmission in the processing of cognitive function. Haloperidol is thought to exert its therapeutic effects through dopamine D₂ receptor blockade because of its strong correlation between clinical efficacy and affinity for dopamine D₂ receptors. However, delay in therapeutic efficacy of haloperidol, like other neuroleptics, is puzzling. Haloperidol-mediated dopamine D₂ receptor blockade occurs within hours but antipsychotic effects take several days or weeks to develop. Activation of the extracellular signal-regulated kinase (ERK) pathway and immediate early genes in response to haloperidol treatment suggests participation of protein synthesis machinery in this process. Changes in the protein level of specific receptor types may contribute to delayed therapeutic efficacy of haloperidol because drug-mediated modulation in protein synthesis is expected to happen much later than the D₂ receptor blockade.

Here, we have tested the theory of whether there is an imbalance in receptor neurotransmission due to a change in receptor levels and its correlation with the cognitive status in brains of young and aged nonhuman primates chronically treated with haloperidol. Both the striatum and prefrontal cortex areas of the brain of the haloperidol-treated animals with significant cognitive loss were processed for the determination of levels of various receptors associated to G-protein signaling and ion channels. Our results show that there was selective abnormality in dopaminergic receptor function in both areas that correlated with the cognitive profiles of both young and aged group of monkeys.

Materials and methods

Materials

Radioligands [¹H]SCH 23390, [¹H]Raclopride, [¹H]Flunitrazepam, [¹H]Scopolamine, and [¹H]Ketanserin were purchased from PerkinElmer España SL (Madrid, Spain). The haloperidol (powder form) and antagonists (+) butaclamol-HCl, fluphenazine, benzodiazepine, LY 53857, and metoclopramide were from Sigma-RBI (Madrid, Spain).

Subjects

Nine young Rhesus monkeys aged 5–7 years and eight monkeys aged 18–22 years were used in this study. Animals were maintained in accordance with the guidelines of local Animal Experimentation, Welfare and Protection Committee and European Union Law (Council directive 86/609/EEC adopted in 1986 and the Protocol to the Amsterdam Treaty).

All monkeys were trained on spatial working memory tasks using the Wisconsin General Testing Apparatus (WGTA) according to methods described elsewhere. Animals performing tasks with 75%–85% accuracy (in case of young animals) and 65%–70% accuracy (in case of old animals), with a 5-second delay period were used for the treatments and further studies.

Cognitive behavioral test

Performance on a spatial delayed response task was used to assess the working memory (WM) status of all the animals.
in this study. To execute the spatial delayed response task, one of the two food wells in WGTA was baited in the view of monkey, then both wells were covered with identical plaques and an opaque screen was lowered for 5 seconds. During the delay period, the monkey must hold in mind the spatial location of the baited well to respond appropriately and be rewarded. After the delay, the animal has to move the correct plaque to obtain the reward. Animals were required to perform 30 trials per test session and cognitive statuses were determined from the mean of 90 trials for each group. These values were used to calculate percentage of correct choice for each as shown in Figure 1.

Haloperidol treatment and cognitive status
Haloperidol treatment in nonhuman primates was performed with the use of a technique from previously published articles. Animals that showed significant reduction in performance on the spatial delayed response task after treatment with haloperidol were considered cognitively-deficient.

Five young monkeys were administered daily with a powder form of haloperidol, disguised in fruit, at a dose of 0.07 mg/kg body weight during the first month, which was increased to 0.14 mg/kg body weight in the second month. The cognitive status of these monkeys was monitored by observing the animal performance on the delayed response task as described above. Haloperidol-mediated cognitive deficits emerged between three and four months in all young monkeys. Animals were sacrificed after four months of the initiation of drug treatment, a period sufficient for substantial WM loss to be observed.

In another set of four aged monkeys, haloperidol treatment was initiated by administration of daily doses of 0.20 mg/kg body weight, increased to 0.27, and 0.35 mg/kg body weight at two-week intervals. The latter dose was maintained until the time of sacrifice. Once the cognitive deficit was observed, animals were sacrificed. Four of each young and aged control group monkeys were given fruit only.

Tissue collection
Monkeys were anesthetized with ketamine and atropine for restraint and then euthanized with an overdose of sodium pentobarbital. They were perfused transcardially with Ringer’s solution, followed by immediate removal of the brain. The brains were rapidly dissected on ice into small blocks of dorsolateral prefrontal cortex (area 46) and striatum (caudate-putamen), frozen in liquid N₂, and stored at −80°C.

Membranes preparation
Collected brain tissues were homogenized in Tris-HCl, pH 7.4 containing a cocktail of protease inhibitors (Sigma-Aldrich, St. Con’s, MO) and processed for membrane preparation as described in earlier studies. Briefly, homogenates were centrifuged at 3000 rpm (1075 g) for 10 min in a...
RC5C centrifuge (Sorvall, Foster city, CA). The resultant supernatant was centrifuged at 48, 100 rpm (105,000 g) for 1 h in XL-90 Ultracentrifuge (Beckman, Fullerton, CA) to collect the membrane fraction as a pellet. Membrane fractions were washed twice in 50 mM Tris-HCl, pH 7.4 and finally suspended in the same buffer and aliquoted. Aliquots were stored at −80°C until used. The protein concentration was determined by the Lowry method.

Receptor binding

For the determination of active receptor concentration in both the prefrontal cortex and striatal membranes, receptor-specific radioligand binding assay was performed. 1,3H 123 I power for dopamine D1 receptor, 3H-Raclopride for dopamine D2 receptor, 3H-Flunitrazepam for GABA receptor, 3H-Scopolamine for acetylcholine muscarinic receptor, and 3H-Ketanserin for serotonin 5-HT1 receptor. For the assay, 200 µg of membrane protein was incubated with 0–20 nM of radioligand and 1 hour at 22°C in a total volume of 0.5 mL of 50 mM Tris-HCl (pH 7.4) containing 1 mM EDTA, 5 mM KCI, 1.5 mM CaCl2, 4 mM MgCl2, and 120 mM NaCl. The reaction was stopped by rapid filtration through 0.3% polyethyleneimine-soaked (GF/B filters; Whatman, Flurham Park, NJ) and washed three times with 50 mM Tris-HCl, pH 7.4. The filters were dried and counted for retained radioactivity. Nonspecific binding was determined by performing the assay in the presence of 1000x of either (+) butaclamol-HCl or fluphenazine for both dopamine receptors, benzodiazepine for GABA receptor, LY 53857 for serotonin 5-HT1 receptor, and metoclopramide for muscarinic receptor. Background activity was subtracted from total activity, which was 5%–7%.

Data analysis

Values are presented as mean ± standard error of mean (SEM) of 4–5 animals in each group. Statistical analysis of the physiologic variables was performed using Student’s t-test. A probability value less than 0.05 was considered statistically significant.

Results

Though WM loss due to haloperidol treatment has previously been demonstrated,20 our idea of a behavioral test in the current study was not only to confirm this observation, but also to use it as a strategy for teasing out the relationship between cognitive deficit and receptor function, together with the prefrontal cortex and striatum, areas known to manifest haloperidol-mediated activity. Additionally, inclusion of both young and aged monkey in our study has added further to our understanding of this relationship. We found that response to haloperidol treatment seen at the receptor level in young animals was much different than in aged animals. However, both young and aged groups of animals showed a significant cognitive loss to the treatment of haloperidol, thus its effect was common to both ages (Figure 1). Receptors contributing to the cognitive change may also be common in both ages.

The results from the ligand binding based estimation of active receptor concentration suggest that chronic haloperidol treatment to both adult and aged monkeys produces significant alterations in receptor-mediated intracellular signaling processes in the striatum and prefrontal cortex. These areas of brain are known to be linked to the antipsychotic effects of haloperidol. In general, the effect of haloperidol treatment in aged monkeys was restricted to dopamine receptors. In the young group of animals, however, the effect was spread to many receptors. We have observed that in the prefrontal cortex, dopamine D1 receptor level was reduced by 32.8% ± 1.6% in young monkeys and 28.4% ± 2.4% in aged monkeys after receiving the treatment with haloperidol (Figure 2A). However, in contrast to dopamine D1 receptors, haloperidol treatment induced increase in dopamine D2 receptor levels (Figure 2B). The haloperidol-mediated increase in D2 receptor was 33.1% ± 3.1% in young monkeys and 31.5% ± 3.9% in aged monkeys (Figure 2B). In the same area, there was a marginal increase in GABA receptor levels (Figure 2C), and no effect in muscarinic receptors (Figure 2E), but a robust decrease (37.6% ± 4.1%) in serotonin 5HT receptors (Figure 2D) was observed in haloperidol-treated young animals. Aged monkeys showed no effect in GABA receptor (Figure 2C), serotonin 5HT receptors (Figure 2D), or muscarinic (Figure 2E) receptors after the treatment with haloperidol. With the consideration of cognitive changes seen in the same animals, we observed that in prefrontal cortex, the effect of haloperidol treatment in both young and aged group of animals was confined more to dopamine D1 and D2 receptors (Figure 2).

Dissimilar to the prefrontal cortex, haloperidol treatment produced a distinct profile in the striatum (Figure 3). In both groups of animals, haloperidol-mediated effect was mostly related to dopamine D2 receptors only and not to both dopamine D1 and D2 receptors, as was in case of prefrontal cortex. Though no effect of haloperidol treatment on striatal D1 receptors was observed (Figure 3A), we found a robust increase in D2 receptors of young (28.7% ± 3.1%) and aged (21.2% ± 1.6%) monkeys (Figure 3B). Haloperidol drastically reduced striatal GABA receptor (39.6% ±
Haloperidol and dopamine receptor

Figure 2. Receptor levels in prefrontal cortex of animals treated with haloperidol.

Notes: Though the response of young animals to haloperidol treatment was observed in many of the receptors tested, only dopamine D₁ (Figure 2A) and D₂ (Figure 2B) receptors showed changes in animals of both age groups that correlated well to the cognitive profile (Figure 1) of these monkeys. *Indicates results that are significantly different from their control group. ♦ Indicates results that are significantly different from the young animal control group.

2.0%) (Figure 3C), serotonin 5HT₂ receptor (30.3% ± 1.2%) (Figure 3D), and muscaranic receptor (55.1% ± 5.2%) (Figure 3E) levels in young animals, but no change was observed in these receptors in aged animals.

With respect to the effect of aging on these receptors, we found a decreasing trend in aged monkeys with the exception of GABA_A receptors, where no significant change was observed in both areas of brain (Figures 2 and 3). In the prefrontal cortex and striatum, there was a decline of 14%–16% in dopamine D₁ receptors, whereas, the reduction in dopamine D₂ receptors was 23.6% ± 2.4% and 17.1% ± 2.6%, respectively. A noticeable decrease of 25.6% ± 4.2% in serotonin 5HT₂ receptor was observed in the prefrontal cortex as compared to 17.9% ± 1.2% in the striatum. Prefrontal cortical D₂ receptors and serotonin 5HT₂ receptors seem to be profoundly affected by aging. The most striking observation in the aged group of monkeys was that haloperidol treatment was restricted to the dopamine receptors.

Discussion

WM deficits have been proposed as a stable marker for the illness of schizophrenia since they have been described as one of the most consistently observed symptoms in schizophrenic patients. Ongoing antipsychotic drug administration is a critical component of the current remedy for schizophrenia. We, as well as others, have observed that chronic haloperidol administration induces WM deficit in both young and aged monkeys. It has been shown that this cognitive func-
tion is dependent on intact prefrontal systems and that dysfunction in prefrontal cortex causes schizophrenia. The results of a correlation between cognitive dysfunction and abnormality in the function of dopamine receptors in haloperidol-treated monkeys suggest that dopamine receptor activity may play an important role in cognitive processing and that it may be a key to the schizophrenia. Particularly, in the prefrontal cortex, haloperidol-mediated loss in prefrontal dopamine D1 receptors in animals of both age groups coincides well with their cognitive profiles. This is supported by the observation that haloperidol-induced cognitive loss could be reversed by the activation of dopamine D1 receptors. Thus, it has been suggested that the down-regulation of cortical D1 receptors may be a component of response to haloperidol treatment in the prefrontal cortex. Based on our results, however, it is argued that not only is a decrease in dopamine D1 receptors, but also upregulation in dopamine D2 receptors in the prefrontal cortex crucial for haloperidol-induced antipsychotic activity. In concurrence with this, over-activity of D2 receptor signaling is known to be psychotomimetic, and blocking this receptor plays a key therapeutic role in diminishing positive symptoms in schizophrenia.

In addition to the selective activities in the dopamine receptor system of the prefrontal cortex, dopamine D2 receptor antagonism of antipsychotic drugs and their ability to upregulate striatal D2 receptors is considered one of the cornerstones of the dopaminergic hypothesis of schizophrenia. The observation that antipsychotics bind to and block striatal dopamine D2-like receptors in direct correlation with their clinically effective antipsychotic doses suggests that striatal dopaminergic systems play a major role in schizophrenia.

**Figure 3** Receptor levels in the striatum of animals treated with haloperidol.

**Notes:** In contrast to the prefrontal cortex, the effect of haloperidol treatment on the striatum was associated with dopamine D2 receptors (Figure 3B) and not the D1 receptors (Figure 3A) in animals of both age groups. However, only in the young group of animals was a considerable change observed in the other receptors tested. *Indicates results that are significantly different from their control group. ♦Indicates results that are significantly different from the young animal control group.
A number of animal studies report an upregulation of striatal D₂-like receptors after subchronic short-term drug administration,⁴³-⁴⁵ the period during which beneficial clinical effects of antipsychotics on human patients are first noticed. The classic or ‘typical’ antipsychotics, including haloperidol, although effective for blocking psychoses, also cause extrapyramidal movement disorders, both transient (eg, parkinsonism, dystonia, akathisia) and chronic (eg, tardive dyskinesia, TD).⁴⁶-⁴⁷ Atypical antipsychotic drugs, the prototype of which is clozapine, have been developed that are devoid of these extrapyramidal side effects. Short treatment periods of rats with clozapine has been reported not to increase striatal D₂-like receptor levels.⁴⁵,⁴⁸ This suggests that the upregulation of striatal D₂-like receptors is more likely associated with drug-induced extrapyramidal motor side effects rather than mediation of their antipsychotic action.

In addition, never-medicated subjects with schizophrenia sometimes exhibit motor deficits resembling those of patients with primary striatal dysfunction,⁴⁹,⁵⁰ and clinical improvement of schizophrenic symptoms treated with neuroleptics has been found to be associated with increased basal ganglia metabolism, possibly reflecting neuroadaptation processes in the striatum.⁵¹,⁵² It has been shown that chronic, but not acute haloperidol treatment facilitates the emergence of long-term potentiation (LTP) versus long-term depression (LTD) at cortico-striatal excitatory synapses. The D₃L isoform of D₂ receptors is responsible for the facilitatory action of classical antipsychotics on excitatory transmission in the striatum. It is known that cortical excitatory information is converged to the striatum at a constant pace, where it modulates local cellular activity. Considering our observation of down-regulation of dopamine D₂ receptor activity and inhibition of glutamate N-methyl D-aspartate (NMDA) receptor function due to upregulation in D₂ receptor in prefrontal cortex, it is suggested that not only D₁-mediated but also NMDA-mediated excitatory input in the striatum is significantly reduced in these animals, causing an overall striatal dysfunction. Therefore, in addition to the higher dopamine D₂ receptor activity in the striatum that is expected to promote stronger inhibition at the excitatory input site, it is likely that an aberration in dopamine receptor-mediated excitatory input to the striatum plays a fundamental role in the development of extrapyramidal side effects.

Disclosures
The authors report no conflicts of interest in this work. This work was supported financially by Ministerio de Ciencia e Innovacion grant BFU 06-0306 and a Junta de Andalucia grant CTS 586/08 to Z.U.K.

References
21. Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. 
24. Keck PE Jr, Cohen BM, Baldessarini RJ, McElroy SL. Time course of antipsychotic effects of neuroleptic drugs. 
27. Gerfen CR, Miyachi S, Paletzki R, Brown P. D, dopamine receptor supersensitivity in the dopamine-depleted striatum results from a switch in the regulation of ERK1/2/MAP kinase. 
28. Goldman PS, Rosvold HE, Mishkin M. Evidence for behavioral impairment following prefrontal lobectomy in the infant monkey. 
30. Khan ZU, Gutierrez A, De Blas AL. Short and long form gamma 2 subunits of the GABAA/benzodiazepine receptors. 
32. Khan ZU, Gutierrez A. Distribution of C-terminal splice variant of G alpha i2 in rat and monkey brain. 
34. Sharma T, Modler D. The cognitive efficacy of atypical antipsychotics in schizophrenia. 
35. Fuster JM. Memory in the cortex of the primate. 
  Physiol Behav. 1993;150(9):19–71.
36. Fritts ME, Asbury ET, Horton JE, Isaac WL. Medial prefrontal lesion deficits involving or sparing the prelimbic area in the rat. 
  Physiol Behav. 1998;64(3):373–380.
38. Luchins D. The dopamine hypothesis of schizophrenia. A critical analysis. 
40. Sayed Y, Garrison JM. The dopamine hypothesis of schizophrenia and the antagonistic action of neuroleptic drugs-a review. 
41. Snyder SH. The dopamine hypothesis of schizophrenia: focus on the dopamine receptor. 
42. van Kamen DP. The dopamine hypothesis of schizophrenia revisited. 
43. Burt DR, Creese I, Snyder SH. Antischizophrenic drugs: chronic treatment elevates dopamine receptor binding in brain. 
44. Seeman P. Brain dopamine receptors. 
46.Jeste DV, Wyatt RJ. In search of treatment for tardive dyskinesia: review of the literature. 
  Schizophr Bull. 1979;5(2):251–293.
47. Baldessarini RJ, Tarsy D. Dopamine and the pathophysiology of dyskinesias induced by antipsychotic drugs. 
49. Caligiuri MP, Lohr JB, Jeste DV. Parkinsonism in neuroleptic-naive schizophrenic patients. 
52. Corson PW, O’Leary DS, Miller DD, Andreasen NC. The effects of neuroleptic medications on basal ganglia blood flow in schizophreniform disorders: a comparison between the neuroleptic-naive and medicated states. 