Acute Administration of Haloperidol Does Not Influence ^{123}I-FP-CIT Binding to the Dopamine Transporter

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A recent ^{123}I-FP-CIT (^{123}-I-*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane) SPECT study on rats suggested that a single 1 mg/kg dose of the antipsychotic haloperidol induces enough dopamine release to compete with ^{123}I-FP-CIT for binding to the dopamine transporter. Taking into account the far-reaching consequences of this proposition, we were interested in testing whether we could reproduce this finding using storage phosphor imaging. **Methods:** Twenty rats were pretreated with saline or haloperidol (1 mg/kg of body weight) and then injected with ^{123}I-FP-CIT. Two hours after ^{123}I-FP-CIT injection, the rats were sacrificed and binding in the striatum, nucleus accumbens, and cerebellum (nonspecific binding) was measured. **Results:** In contrast to the earlier SPECT finding, acute administration of haloperidol did not induce a significant change in ^{123}I-FP-CIT binding ratios in the striatum and nucleus accumbens. **Conclusion:** Changes in synaptic dopamine due to acute haloperidol administration were not detectable with ^{123}I-FP-CIT.

Key Words: dopamine transporter; haloperidol; antipsychotics; dopamine release; rats

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The release of dopamine in the brain is critical for an accurate execution of reward- and goal-directed behavior (*1*). Disturbances in dopamine release have been implicated in many neuropsychiatric disorders. Dopamine $D_{2/3}$ receptors are expressed in the cell membrane of dopamine neurons (i.e., autoreceptors) to regulate dopamine release (*2*). An increase of extracellular dopamine concentrations will lead to a stimulation of dopamine autoreceptors, which will dampen the release of dopamine. In this respect, it is of interest that impulsivity may be related to impaired dopamine autoreceptor functioning (*3*). Furthermore, antipsychotics, such as haloperidol, are dopamine $D_{2/3}$ receptor antagonists. As expected, microdialysis studies showed that acute administration of haloperidol enhanced striatal dopamine release in rats (*4*).

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In the last decade, dopamine $D_{2/3}$ receptor imaging has been used extensively to measure dopamine release (*5,6*). Intriguingly, Nikolaus et al. suggested that dopamine transporter (DAT) imaging could also be of value for measuring acute changes in dopamine concentrations (*7*). More specifically, using a small-animal scanner, they reported that acute administration of haloperidol (1 mg/kg) in rats reduced binding of striatal ^{123}I-FP-CIT (^{123}-I-*N*-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane). To explain their findings, the authors argued that haloperidol enhanced dopamine release, which may compete with ^{123}I-FP-CIT for binding to DAT. Taking into account the far-reaching consequences of this proposition, we were interested in seeing whether we could reproduce their finding by using a well-validated, sensitive, and high-resolution technique, namely storage phosphor imaging, for measuring ex vivo radiotracer binding in rat brain (*8*).

MATERIALS AND METHODS

Male Wistar rats (295–310 g) were randomized to intraperitoneal injections of either saline ($n = 10$) or haloperidol (1 mg/kg of body weight; $n = 10$; Janssen Pharmaceuticals, Inc.). One hour later, the rats were anesthetized as previously described (*9*) and administered ^{123}I-FP-CIT (~37 MBq; specific activity > 750 MBq/nmol; GE Healthcare) through the tail vein. The rats were sacrificed 2 h later (*10*), and the brains were removed and sliced as described earlier (*9*). Then, the image plates were exposed for 24 h, and storage phosphor imaging was performed as described earlier (*8,9*). In contrast to previous studies, we used a Typhoon FLA 7000 phosphor imager (GE Healthcare Life Sciences) and Hypercassette imaging plates (Amersham Biosciences), which were scanned at a 25-μm pixel size.

Regions of interest were drawn for the striatum, nucleus accumbens (ventral striatum), and cerebellum (reflecting nonspecific binding) according to a standard brain atlas and analyzed using dedicated software (AIDA Image Analyzer; Raytest) as previously described (*9,11*). We looked into the dorsal and ventral parts (*12*). As the outcome measure, ratios of total to nonspecific binding were calculated for the striatum and nucleus accumbens. Cerebellar binding was used for the assessment of nonspecific binding because ^{123}I-FP-CIT binding cannot be blocked by a DAT blocker in rats (*10*). Because data were normally distributed, 1-way ANOVA was performed to assess between-group differences with a probability value of 0.05.

All procedures were approved by the Animal Ethics Committee of the Academic Medical Center.

RESULTS

Clear ^{123}I-FP-CIT binding was visible in the striatum and nucleus accumbens, both in saline-treated rats and in haloperidol-treated rats (Fig. 1).

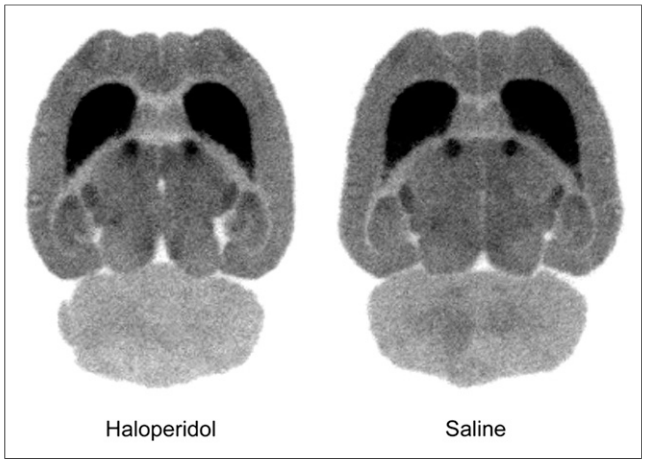

FIGURE 1. Storage phosphor images of haloperidol-pretreated and saline-pretreated rats at level of dorsal striatum. Striatal ^{123}I-FP-CIT binding (expressed as photostimulated luminescence) is intense in both rats and low in cerebellum.

For the dorsal striatum, binding ratios were 3.68 ± 0.26 (average of the 2 sides ± SD) for the saline-treated group and 3.73 ± 0.30 for the haloperidol-treated group. This difference was not significant (P = 0.72; Fig. 2). For the ventral striatum (nucleus accumbens), binding ratios were 2.54 ± 0.05 for the saline-treated group and 2.56 ± 0.11 for the haloperidol-treated group (in 1 animal of this group, the accumbens binding could not be analyzed accurately). This difference was also not significant (P = 0.50; Fig. 2).

DISCUSSION

In this study, we did not find a statistically significant effect of acute haloperidol administration on striatal ^{123}I-FP-CIT binding in rats for either the dorsal or the ventral (nucleus accumbens) parts of the striatum.

It is well documented that acute administration of haloperidol can increase the striatal dopamine concentration (*4*). The question remains, however, of whether one can theoretically predict that the dopamine release induced by an acute single 1 mg/kg dose of haloperidol (as executed in the present experiment as well as in the study by Nikolaus et al. (*7*)) is high enough to induce changes in ^{123}I-FP-CIT binding to DAT. A previous study showed that acute administration of a 1 mg/kg dose of haloperidol induces a relatively slow and small increase in extracellular dopamine, namely no more than 50% of baseline levels (*13*). In this regard, it is of interest that striatal dopamine $D_{2/3}$ receptor imaging is a well-validated method to assess striatal dopamine release (*6*). Applying this method shows that a 4- to 5-fold increase (400%–500%) of extracellular dopamine is required to produce a decrease of only 10% in striatal dopamine $D_{2/3}$ receptor binding (*14*). Similarly, effects of dopamine on the DAT expression are reported only after administration of high doses of dopamine. Richards and Zahniser showed significant decreases in DAT cell surface expression of 49% in the dorsal striatum and 22% in the nucleus accumbens after a 1-h preincubation period with 100 μM dopamine (*12*). However, in monkeys, the baseline striatal extracellular dopamine concentration is around 5–6 nM (*14*). So, if the increase induced by acute administration of a 1 mg/kg dose of haloperidol is indeed not more than 50%, the increase of dopamine may be only 2–3 nM. If one assumes that there is a linear relationship between DAT downregulation and dopamine concentration, it is not likely that such a small increase in dopamine concentrations may lead to large changes in DAT levels. In line with this possibility, we did not observe even a trend for a change in DAT binding in the dorsal striatum, an area that may be more prone to downregulation of DAT than the ventral striatum (*12*). In addition, Hadlock et al. showed that sole administration of the dopamine D_2 antagonist eticlopride (0.5 mg/kg intraperitoneally) also did not influence DAT expression in rats (*15*). Finally, the binding of ^{123}I-FP-CIT to DAT is nearly irreversible (*16*), strongly implying a very small dissociation constant, which in turn means that dopamine would have to be present at huge concentrations to displace it. All in all, although it is well accepted that DAT substrates, including high dopamine concentrations, may influence DAT binding in vivo, our present data do not support the possibility that acute administration of a single 1 mg/kg dose of haloperidol induces enough dopamine release to influence ^{123}I-FP-CIT binding in rats.

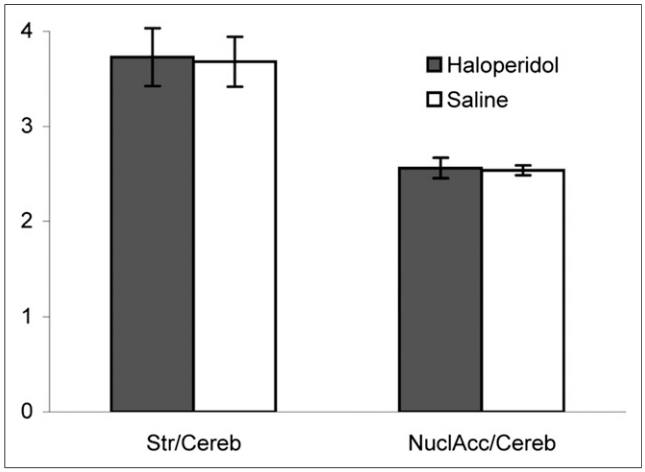

FIGURE 2. ^{123}I-FP-CIT binding ratios for striatum to cerebellum and nucleus accumbens (ventral striatum) to cerebellum for saline- and haloperidol-treated rats (mean ± SD).

Nikolaus et al. argued that the haloperidol-induced dopamine release may compete with ^{123}I-FP-CIT binding (*7*). After dopamine is released, it is rapidly taken up as a substrate via DAT (*17*). The rate of dopamine uptake is described by the Michaelis–Menten equation ($d[dopamine]/dt = -V_{max}[K_m[dopamine] + 1)$ (*18*). In this equation, K_m is the Michaelis–Menten constant for uptake, V_{max} the uptake rate, and d[dopamine]/dt the rate of disappearances of dopamine. When the dopamine concentration is sufficiently high, this uptake can be saturated. However, when there are low increases in the concentration of extracellular dopamine, the DAT uptake is unsaturated, and dopamine disappears by a first-order process with a rate constant of V_{max}/K_m (*18*). Consequently, it is questionable whether acute dopamine release induced by a single 1 mg/kg dose of haloperidol can compete with DAT binding.

We tried to imitate several relevant parts of the study by Nikolaus et al. (*7*) as much as possible. Haloperidol and saline were administered intraperitoneally 1 h before injection of the radiotracer in Wistar rats, and at the same dose. Also, the same anesthetics were used, and DAT binding was assessed 2 h after injection, a time point at which a pseudoequilibrium is reached in rats (*10*). We also used the same DAT radiotracer. One difference

is that we used saline in the control group instead of ethanol. However, Nikolaus et al. used haloperidol from Sigma-Aldrich. In this formulation, ethanol is used as a solvent. We used haloperidol produced by Janssen Pharmaceuticals, Inc. In this formulation, ethanol is not used as a solvent, and we therefore used saline to pretreat the animals in the control group. We do not believe that this difference can explain the conflicting results. An explanation of why we could not replicate a significant effect of haloperidol on striatal ^{123}I-FP-CIT binding in rats might be as follows. In the Nikolaus study, SPECT was performed on the Tier-SPECT. However, accurate delineation of striatal DAT binding is more difficult than with storage phosphor imaging, and it may be even harder to delineate nonspecific binding in the cerebellum. In their study, the SPECT assessments were not validated with ex vivo measurements. We used a technique (*8*) in which dorsal striatal, nucleus accumbens, and cerebellar binding can be delineated easily (Fig. 1), resulting in high binding ratios with a small SD within groups.

A limitation of our study was the relatively small groups that were studied. However, the difference between groups did not show even a trend toward statistical significance (the striatal binding ratios were even 1% higher in the haloperidol-treated rats than in the saline-treated rats; Fig. 2). Also, a positive control group was lacking; however, in a previous study we showed that the DAT blocker methylphenidate was able to decrease the ^{123}I-FP-CIT binding in rats using storage phosphor imaging (*8*). Finally, only a single dose of haloperidol was tested. Higher doses of haloperidol (e.g., 5 mg/kg) may be able to influence DAT binding, at least in rats (*19*). However, the results of such high doses are of less importance when one is considering whether it's worth evaluating if a single dose of haloperidol is able to induce enough dopamine release in humans.

CONCLUSION

Changes in synaptic dopamine due to acute haloperidol administration were not detectable using the SPECT tracer ^{123}I-FP-CIT. It is therefore unlikely that ^{123}I-FP-CIT SPECT in combination with acute administration of haloperidol in a tolerable dose can be used to detect changes in synaptic dopamine release in vivo in humans.

DISCLOSURE

The costs of publication of this article were defrayed in part by the payment of page charges. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734. Jan Booij is a consultant at GE Healthcare. No other potential conflict of interest relevant to this article was reported.

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