

Ovariectomy Results in Lower Plasma Haloperidol Levels in Rats Following Chronic Administration

Jeffrey W. Grimm,¹ Manickam Aravagiri,² and Ronald E. See^{1,3}

Received May 5, 1998; accepted July 15, 1998

KEY WORDS: haloperidol; plasma; ovariectomy; estrogen; therapeutic drug monitoring.

INTRODUCTION

Therapeutic drug monitoring of antipsychotic drugs (APDs) has been argued to be beneficial in providing enhanced drug efficacy with minimal side effects (1). We have previously investigated neurochemical and behavioral effects of chronic APD administration (≥ 6 months) in gonadally intact rats and have reported plasma levels of haloperidol after chronic exposure as an end point measure (2–4). The data described in this short report refer to plasma haloperidol levels derived from similarly treated rats, except that both ovariectomized and gonadally intact female rats were utilized. Haloperidol was chosen as it is a commonly prescribed APD that has a moderate to high liability to produce extrapyramidal motor side effects. Ovariectomy was chosen as a variable because there is evidence of a lower incidence and later onset of schizophrenia in women (5) as well as a higher propensity to develop these motor side effects following APD treatment (6). It has been argued that these sex differences relate to circulating ovarian hormones such as estrogen (5). As detailed in this report, plasma haloperidol levels were found to be markedly lower in ovariectomized rats.

METHODS

The animals utilized in this study were cared for in compliance with *Principles of Laboratory Animal Care* (NIH publication #85–23, revised 1985) and the experimental protocols were approved by the Washington State University Institutional Animal Care and Use Committee. Female, Sprague Dawley rats were ovariectomized by bilateral flank incision or given sham surgeries at the age of 3 months (initial mean weight = 321 ± 5 g). Twelve days later, rats began to receive haloperidol (0.02 mg/ml) or vehicle treated drinking water. Haloperidol and vehicle solutions were made fresh weekly (3). Briefly, haloperidol drinking water was prepared by first dissolving 80 mg of haloperidol (SIGMA, St. Louis, MO, USA) in 1 ml glacial acetic acid. This solution was then added to 4 L tap water with 2 ml

of 1N NaOH and 4 ml of 70% dextrose for flavoring. Vehicle drinking water was prepared with all constituents, except for haloperidol. Both solutions were at approximately pH 6. Throughout the course of the study, biweekly solution intake was recorded. After 24 weeks of administration and 3 days after withdrawal from haloperidol or vehicle solutions, rats were rapidly decapitated and trunk blood was collected. Blood samples were collected into heparinized glass tubes (5 ml), immediately centrifuged ($2,500 \times g$ for 5 min), and plasma was collected and frozen at -70°C . Plasma samples (1 ml plasma plus 0.5 ml saturated sodium carbonate with 4 ng of chlorohaloperidol as an internal standard) were later analyzed by high performance liquid chromatography for haloperidol and reduced haloperidol using previously described methods (chlorohaloperidol, haloperidol, and reduced haloperidol standards from R.B.I., Natick, MA, USA) (7). Briefly, aliquots of samples (extracted with 7 ml 10% methylene chloride, evaporated under nitrogen at 55°C , and then reconstituted in 150 μl acetonitrile) were injected onto an Ultrasphere cyano column (25 cm \times 0.46 cm I.D., 5 μm ; Beckman, San Ramon, CA, USA). Mobile phase consisted of 0.04 mol/l ammonium acetate (pH 6.8)-methanol-acetonitrile (8:6:86, v/v) and was degassed and filtered (0.2 μm) prior to use. Detection of analytes was accomplished with a Coulochem detector Model 5100A (ESA, Bedford, MA, USA) connected to a high sensitivity analytical cell (Model 5011, ESA) and guard cell (Model 5020, ESA). Electrode settings were as follows: analytical cell, +0.6 and +0.95 V (electrode 1 and 2); guard cell, +1.0 V. The lower limit of quantitation for analyte levels was 0.1 ng/ml.

RESULTS

Three way (drug \times gonadal status \times time) repeated measures analysis of variance was utilized to statistically examine body weight and solution intake data over the course of the 6 month study. Plasma levels of haloperidol and reduced haloperidol on the day of sacrifice were compared using a t-test. For clarity, only measures relating to the day of sacrifice when plasma was obtained are presented in Table 1. Over the course of the chronic study, control water treated rats significantly differed according to gonadal status for both water consumption and body weight, with sham ovariectomized rats consistently drinking more water and ovariectomized rats consistently weighing more. In contrast, water consumption and body weights for rats treated with haloperidol did not differ according to gonadal status. When converted to average dose (mg/kg/day) of haloperidol, haloperidol-treated rats did not differ according to gonadal status. Body weights on the final day of the experiment (day of sacrifice) showed the same between group relationships, again with both haloperidol-treated groups retaining equivalent mean body weights (Table 1). In sum, rats treated with chronic oral haloperidol, regardless of gonadal status, received equivalent amounts of drug. Statistical analysis of plasma levels of haloperidol, however, revealed a significant difference between gonadally intact and ovariectomized rats. Ovariectomized rats had approximately 50% of the amount of plasma haloperidol as was measured in sham ovariectomized rats. As indicated in Table 1, this difference remained even when the data was analyzed without 3 data points measured as non-detectable (and therefore assigned a value of zero) in the

¹ Department of Psychology and Program in Neuroscience, Washington State University, Pullman, Washington 99164-4820.

² Neuropsychiatric Institute, University of California, Los Angeles, California 90024.

³ To whom correspondence should be addressed. (e-mail: see@mail.wsu.edu)

Table 1. Means \pm SEMs of Consumption, Weights, and Plasma Analyte Levels at Time of Sacrifice^a

| | CON/SHAM N = 12 | CON/OVX N = 13 | HAL/SHAM N = 8 | HAL/OVX N = 10 |
|--|----------------------|----------------------|--------------------|--------------------|
| Consumption (ml/kg/day) | 146.17 \pm 3.86*** | 109.37 \pm 3.06*** | 76.98 \pm 3.10 | 78.57 \pm 2.69 |
| Haloperidol dose (mg/kg/day) | — | — | 1.54 \pm 0.06 | 1.57 \pm 0.05 |
| Body weight (g) | 383.50 \pm 10.04 | 430.92 \pm 12.47* | 355.00 \pm 14.20 | 357.40 \pm 11.59 |
| Plasma haloperidol (ng/ml) | — | — | 0.47 \pm 0.07*** | 0.18 \pm 0.05 |
| Plasma haloperidol with NDs removed ^b | — | — | 0.47 \pm 0.07* | 0.26 \pm 0.06 |
| Plasma reduced haloperidol (ng/ml) | — | — | 44.20 \pm 12.64 | 37.89 \pm 9.68 |

^a SHAM or OVX denotes sham ovariectomy or ovariectomy; CON or HAL denotes treatment with vehicle solution or haloperidol solution. Significant difference from other group(s) are indicated: * $p < 0.05$, *** $p < 0.001$.

^b Three rats in the HAL/OVX group had values of plasma haloperidol at non-detectable levels (ND). Plasma levels were therefore compared between groups using zeros for these values and with the NDs removed.

ovariectomized group. In contrast to haloperidol levels, no significant differences were noted in plasma levels of reduced haloperidol.

DISCUSSION

These data suggest that ovarian hormones profoundly influence plasma levels of haloperidol following chronic oral drug administration. While it cannot be determined from the current results, the observed difference may have been due to differential enzymatic activity (e.g., cytochrome P450 isozymes) in the two groups. Other possibilities include differential binding of haloperidol by plasma proteins or differential compartmental distribution of the drug (i.e. greater sequestering of haloperidol in compartments other than plasma in the ovariectomized rats). In any case, the presence or absence of ovarian hormones clearly influenced available levels of haloperidol. These results are, to our knowledge, the first to describe gonadally mediated plasma levels of haloperidol in chronically treated rats. Finding these results in a chronic paradigm are particularly important, as APDs are most commonly administered over the course of several years in clinical settings.

As shown in Table 1, concentrations of reduced haloperidol did not differ according to gonadal status. Reduced haloperidol is a metabolic product of haloperidol that has been shown to have only moderate activity compared to the parent compound in animal and human studies (8). The present results indicate that ovarian hormones may have a modulatory role over haloperidol specific to the parent compound. Alternatively, as haloperidol levels were higher in ovariectomized rats (yet reduced haloperidol levels were equivalent between groups), ovarian hormones may facilitate clearance of reduced haloperidol.

Estrogen is the gonadal hormone most likely to have an influence on APD effects. For example, there is evidence of "neuroleptic-like" effects of estrogen which have been proposed to account for the lower incidence and later onset of schizophrenia in women (5). Specifically, estrogen is argued to mediate dopamine transmission, which is the same neurotransmitter affected by APDs like haloperidol (9). Preclinical studies in animals have generally supported this hypothesis with findings of significant estrogen/dopamine interactions. For example, estrogen has repeatedly been shown to result in alterations in dopamine levels and dopamine receptors in the striatum (10) and ovariectomized rats show a persistent increase in striatal dopamine receptors (11).

While such hormone/dopamine-mediated effects may account for some of the sex-related differences in schizophrenic symptoms, it is possible that direct hormonal interactions with the APD used to treat psychosis can also account for some of these differences. By increasing plasma haloperidol (and presumably increasing brain levels of haloperidol), the presence of ovarian hormones could enhance both the antipsychotic effects and motor side effect liability of haloperidol. Clinical evidence supports such a hypothesis in that women generally require lower dosages of haloperidol than men (12) and some forms of motor side effects appear to be more prevalent in women (6). In the context of the present findings, it may be suggested that females treated with haloperidol might be titrated to significantly lower, yet still effective, doses of haloperidol than males. Conversely, post-menopausal women may actually require higher doses than younger counterparts. Indeed, there is evidence of higher APD dose requirements in middle-aged women as compared to similarly aged men (13). Further exploration of the interaction of ovarian hormones with other APDs is warranted.

ACKNOWLEDGMENTS

This research was supported by PHS grant DE09678.

REFERENCES

1. R. Eilers. Therapeutic drug monitoring for the treatment of psychiatric disorders. Clinical use and cost effectiveness. *Clin. Pharmacokinet.* **29**:442-450 (1995).
2. R. E. See, M. A. Chapman, C. E. Murray, and M. Aravagiri. Regional differences in chronic neuroleptic effects on extracellular dopamine activity. *Brain Res. Bull.* **29**:473-478 (1992).
3. R. E. See and M. A. Chapman. Chronic haloperidol, but not clozapine, produces altered oral movements and increased extracellular glutamate in rats. *Eur. J. Pharmacol.* **263**:269-276 (1994).
4. R. E. See, A. M. Lynch, M. Aravagiri, C. B. Nemeroff, and M. J. Owens. Chronic haloperidol-induced changes in regional dopamine release and metabolism and neurotensin content in rats. *Brain Res.* **704**:202-209 (1995).
5. M. V. Seeman and M. Lang. The role of estrogen in schizophrenia gender differences. *Schizophr. Bull.* **16**:185-194 (1990).
6. W. M. Glazer, F. Naftolin, D. C. Moore, M. Bowers, and N. J.

- MacLusky. The relationship of circulating estradiol to tardive dyskinesia in men and post-menopausal women. *Psychoendocrinology* **9**:429-434 (1981).
7. M. Aravagiri, S. R. Marder, T. Van Putten, and B. D. Marshall. Simultaneous determination of plasma haloperidol and its metabolite reduced haloperidol by liquid chromatography with electrochemical detection. Plasma levels in schizophrenic patients treated with oral or intramuscular depot haloperidol. *J. Chromatogr. Biomed. Appl.* **656**:373-381 (1994).
 8. Y. W. Lam, W. Chang, M. W. Jann, and H. Chen. Interindividual variabilities in haloperidol interconversion and the reduced haloperidol/haloperidol ratio. *Neuropsychopharmacology* **7**: 33-39 (1992).
 9. L. Farde, F. A. Wiesel, C. Halldin, and G. Sedvall. Central D₂-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. *Arch. Gen. Psychiatry* **45**:71-76 (1988).
 10. T. Di Paolo. Modulation of brain dopamine transmission by sex steroids. *Rev. Neurosci.* **5**:27-42 (1994).
 11. J. Z. Fields and J. H. Gordon. Permanent haloperidol-induced dopamine receptor up-regulation in the ovariectomized rat. *Brain Res. Bull.* **26**:549-552 (1991).
 12. R. J. Dworkin and G. L. Adams. Pharmacotherapy of the chronic patient: Gender and diagnostic factors. *Community Ment. Health J.* **20**:253-261 (1984).
 13. M. V. Seeman. Interaction of sex, age, and neuroleptic dose. *Comprehensive Psychiatry* **24**:125-128 (1983).