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# Table of Contents

Abstract ........................................................................................................................................ 2
Introduction .................................................................................................................................. 3
Background .................................................................................................................................. 3
  Antipsychotic Medication: Mechanisms of Action ................................................................. 4
  Prolactin: Anatomy and Physiology ......................................................................................... 6
  Relationship to Dopamine Antagonism .................................................................................... 8
  Antipsychotics and Prolactin: Chronic Effects ....................................................................... 8
  Antipsychotics and Prolactin: Acute Effects ......................................................................... 10
Objective ..................................................................................................................................... 13
Hypothesis .................................................................................................................................. 13
Methods ...................................................................................................................................... 13
  Procedure ................................................................................................................................. 14
Data Analysis ............................................................................................................................... 15
Results .......................................................................................................................................... 16
  Control Data ............................................................................................................................ 17
Discussion .................................................................................................................................... 18
Further Considerations ................................................................................................................ 21
Future Directions and Conclusions ............................................................................................ 22
References .................................................................................................................................... 23
Appendix A
  Table 1 ...................................................................................................................................... 28
  Tables 2a-d ................................................................................................................................. 29
Appendix B
  Figure 1 ...................................................................................................................................... 33
  Figures 2a-c ................................................................................................................................. 34
  Figures 3a-e ................................................................................................................................. 37
Abstract

Introduction: Evidence suggests that, unlike typical antipsychotics, 'atypicals' spare the prolactin (PRL) secreting system. Most of the evidence, however, is based on studies of single-point PRL levels, usually done 12-24 hours post drug dose. The kinetics of PRL response over the course of a single dose cycle with atypical antipsychotics has not been heretofore examined. Hypothesis: All antipsychotic medications studied will give rise to some degree of prolactin elevation over the course of a single dose cycle. Methods: PRL levels in 18 male patients with schizophrenia maintained on their current antipsychotic for ≥ 4 weeks were monitored via an indwelling intravenous catheter over 24 hours following an oral dose of their daily medication. Results: Patients in each drug group demonstrated an increase in PRL levels above their baseline values. Conclusions: These data demonstrate that all tested atypical antipsychotics give rise to PRL elevation after each dose, even after chronic dosing previously.
Introduction

The introduction of clozapine into clinical practice was a significant breakthrough in the treatment of psychosis as it was proved effective in treating patients formerly resistant to the commonly used antipsychotics previously available. Subsequent research demonstrated that clozapine, unlike other antipsychotics, demonstrates unique clinical properties, such as lack of prolactin elevation and no significant extrapyramidal side effects (EPS) (Kane et al., 1988). These unique clinical features led researchers to classify clozapine as an "atypical" antipsychotic. Over the past two decades, new antipsychotic medications which maintain the atypical clinical profile of clozapine, -ie., no prolactin elevation and lack of EPS - have continued to be developed. It has become increasingly evident, however, that antipsychotics classified as atypical may not completely spare the prolactin system. Human and rodent data have shown that the acute administration of clozapine does increase prolactin levels (Meltzer et al., 1975; 1979), adding complexity to the atypical antipsychotic/prolactin issue.

Background

Antipsychotic Medication: Mechanisms of Actions

The dopamine hypothesis of schizophrenia posits that the manifestation of this disorder is the result of underlying hyper-dopaminergic activity in critical brain regions (Van Rossum, 1967). The hypothesis was supported by the finding that symptoms of psychosis could be attenuated by antipsychotic medications acting as dopamine receptor antagonists (Van Rossum, 1967). Various pieces of evidence linking dopamine to the pathophysiology of schizophrenia have emerged over time (Seeman, 1987). One of the strongest pieces of evidence in support of this hypothesis is that the clinical potency of antipsychotics is highly correlated with their in vitro binding affinity for the dopamine D2 receptor (Seeman & Lee, 1975), a relationship which is not observed for serotoninergic, histaminergic, muscarinic, adrenergic or other dopamine receptor systems (Peroutka & Snyder, 1980).
It has been shown as well that both the beneficial and adverse clinical effects of antipsychotics correlate with the proportion of striatal dopamine D2 receptors occupied (Farde et al., 1993; Kapur et al., 1996). Numerous in vivo studies using positron emission tomography (PET) and single photon emission tomography (SPECT) have found a relationship between percent striatal dopamine D2 receptor occupancy and clinical parameters such as improvement of psychotic symptoms, degree of EPS and hyperprolactinemia (Farde et al., 1988; Kapur et al., 1996; 2000).

Measurable clinical improvement generally occurs when at least 60% of striatal dopamine D2 receptors are occupied by antipsychotics (Farde et al., 1988, 1992 & Nordstrom et al, 1993). For example, a PET study with 11C-raclopride reported that patients on typical antipsychotics with striatal D2 receptor occupancies between 65% and 85% demonstrated clinical improvement (Farde et al., 1988). Subsequent PET studies have confirmed a relationship between significant clinical improvement and receptor occupancy. Farde and others (1992) found that all 27 patients who had a striatal dopamine D2 receptor occupancy above 70% were rated as being “much improved” or “very much improved” based on the Clinical Global Impression Scale (CGI). Moreover, Nordstrom and others (1993) have also found that clinical outcome measured by the CGI was more favourable in 17 patients with receptor occupancies between 60% and 80% than in those with lower occupancies. In a recent double-blind PET study in 22 patients with first-episode schizophrenia, Kapur and others (2000) provide further support for this observation, reporting that dopamine D2 receptor occupancy above 65% was associated with moderate to high clinical response based on the CGI. No absolute threshold for clinical response was found in this or the aforementioned studies, although this group did find that 80% of responders were above 65% dopamine D2 receptor occupancy while 67% of non-responders were below it.

Looking at atypical agents at clinically effective doses, clozapine has a mean striatal D2 receptor occupancy of only 47% (range 20%-67%) with a very high level of 5-HT2 occupancy (84%-94%) (Nordstrom et al., 1995). Farde et al. (1992) and Kapur et al. (1999) have also
confirmed that clozapine, at therapeutic doses, gives rise to a low dopamine D₂ receptor occupancies of 47% (n=5) and 52% (n=11), respectively. Olanzapine, at clinically therapeutic doses, produces D₂ receptor occupancy ranging between 43%-89% (n=17), with a high degree of 5-HT₂ blockade (over 90%) (Kapur et al., 1999). At clinically effective doses of 3 to 6 mg/day, risperidone produces D₂ receptor occupancy ranging between 53%-85% also with a very high degree of 5-HT₂ blockade (Nyberg et al., 1999). These results are similar to those reported by Kapur et al. (1999), in which they found that 13 patients treated with 2 to 6 mg/day of risperidone demonstrated D₂ receptor occupancies ranging between 63%-85% (M=74%).

The same data also suggest that development of (EPS), an untoward effect of striatal D₂ dopamine receptor occupancy, begins at approximately 80%. Farde et al. (1992) reported that those patients who developed EPS, as measured by both the Simpson-Angus Rating Scale for Extrapyramidal Side Effects and Barnes Akathisia Scale, were those who had an average receptor occupancy level of 82%. Several other studies have provided support for the observation that EPS onset is associated with increased receptor occupancy based on the aforementioned and other rating scales (Knable et al., 1997; Nordstrom et al, 1993; Scherer et al., 1994; Schlosser et al., 1997). Kapur et al. (2000), have found that in 22 patients with first-episode schizophrenia, EPS as measured by the Extrapyramidal Symptom Rating Scale (ESRS), only developed when more that 78% of striatal dopamine D₂ receptors were occupied.

One other clinical parameter which has been found to be related to striatal dopamine D₂ receptor occupancy is hyperprolactinemia (>20 ng/ml) (Daskalakis et al., 1998, Nordstrom et al., 1998). This is a potentially serious side effect in that elevated prolactin levels can lead to sexual dysfunctions in both sexes, amenorrhea in women and, via estrogen reduction, to bone density loss and increased risk of cardiovascular disease after many years of treatment. Unlike clinical improvement and EPS, however, it is still not clear whether there is a significant threshold of D₂ occupancy above which hyperprolactinemia will emerge.

Daskalakis et al. (1998) studied 36 first-episode patients being treated with haloperidol and calculated that most prolactin elevations over what is considered the upper range of normal
(>20 ng/ml for males, >30 ng/ml for females) developed after 75% of striatal D₂ receptors were
blocked. These data were obtained by first calculating an estimate of dopamine D₂ receptor
occupancy from haloperidol plasma levels and then correlating these predicted values with each
subject's plasma prolactin levels. A lower percentage of dopamine D₂ receptor occupancy was
obtained by Nordstrom and others (1998). In a study of 13 patients with schizophrenia, this
group found that hyperprolactinemia (>20 ng/ml for both sexes) emerged in raclopride-treated
patients with as little as 50% striatal D₂ receptor occupancy as measured with PET. Others,
however, have not been able to find a correlation between plasma prolactin levels and D₂
receptor occupancy using both PET (n=25) (Baron et al., 1989) and SPECT (n=12) (Schegel et
al., 1996). Different factors may account for the contradictory results. Subjects in these latter
studies had been treated by a number of different antipsychotics (i.e., typicals and atypicals) via
different routes of administration (i.e., oral or depot), had been on more than one antipsychotic,
or had been taking other psychotropic medications known to affect the prolactin secreting
system. Each of these could obscure a relationship that might otherwise have existed.

Prolactin: Anatomy and Physiology

Prolactin is a naturally occurring hormone which is involved principally in the regulation
of lactation. Prolactin is predominantly found within lactotrophic cells in the pars distalis
portion of the anterior pituitary gland, but extrapituitary sites of prolactin synthesis have also
been reported. Lactotrophs are acidophilic cells of two types. Some lactotrophs are large and
correspond to the densely granulated (granules 250-800 nm in diameter) elongated cells
distributed throughout the gland, whereas others, are small- to medium-sized and correspond to
sparsely granulated (granules 200-350 nm in diameter) cells. These are found along the border
between the lateral wings and the median wedge and in the posterolateral edges of the gland
(Molitch, 1995).

Gender differences regarding prolactin anatomy and physiology have been observed.
Females have both higher basal prolactin levels and demonstrate more robust prolactin increases
than men in response to stress, thyrotropin-releasing hormone (TRH) and psychotropic medications such as antipsychotics (Daskalakis et al., unpublished). Moreover, prolactin levels are known to fluctuate during the menstrual cycle. It has been demonstrated that some, but not all, females have higher prolactin levels at midcycle and lower levels in the follicular stage of the luteal phase (Molitch, 1995). It has been suggested that this may be mediated by estrogen. Estrogen has been shown to increase the number of lactotrophic cells in the anterior pituitary, to decrease hypothalamic dopamine levels, and to reduce lactotroph cell response to dopamine (Daskalakis et al., unpublished).

Prolactin has multiple actions. In women, prolactin is principally involved in preparing the breast for lactation and in some aspects of gonadal function. Substantially less is known about the role of prolactin in men, although it has been implicated in the control of libido, possibly through testosterone modulation (Molitch, 1995).

Prolactin secretion follows a predominant diurnal rhythm which is sleep dependent (Figure 1) (Molitch, 1995; Sassin et al., 1972). Prolactin levels do not rise until sleep begins in subjects who are kept awake to reverse the sleep-waking cycle (Molitch, 1995). Prolactin is secreted at an interpulse interval of approximately 8 minutes, with about 4-14 secretory episodes per day. In drug-free individuals, the amplitude of prolactin secretory pulses increases 60 to 90 minutes after the onset of sleep. Plasma prolactin levels peak during non-rapid-eye-movement (non-REM) sleep and fall during rapid eye movement (REM), reaching a maximum 1 to 2 hours before waking.

In schizophrenia, some evidence suggests that while drug-free males with schizophrenia (n=9) have prolactin levels comparable to age-matched drug-free males (n=9) while awake, prolactin secretion appears to be hyperresponsive to the physiological stimulus of sleep onset (Van Cauter et al., 1991). In drug-free male patients with schizophrenia, there is almost a threefold enhancement of the sleep-related increase in plasma prolactin levels, associated with an intensified frequency of nocturnal prolactin pulses compared to normal male controls (Van Cauter et al., 1991). While the reasons for this are not clear, dysregulation of the serotonergic
system has been suggested as being a contributing factor as the sleep-related prolactin release is, at least partially, under serotonergic control (Mendelson et al., 1975). It is not well known whether a similar prolactin secretory profile is found in drug-free females with schizophrenia.

**Prolactin: Relationship to Dopamine Antagonism**

Prolactin secretion is mainly under the control of dopamine within the diencephalon. Diencephalic dopaminergic cell groups are divided into two different systems: 1) the tuberoinfundibular system which projects from the arcuate nucleus and adjacent periventricular nucleus of the hypothalamus to the median eminence (Molitch, 1995) and 2) the tuberhypophyseal system which projects to the neural and intermediary lobes of the pituitary (Molitch, 1995).

Once tuberoinfundibular (TIDA) neurons are activated, dopamine is released and travels through fenestrated capillaries into the perivascular spaces of the primary plexus (Molitch, 1995). Dopamine is then transported to the anterior pituitary through hypophyseal portal blood where it binds to dopamine D₂ receptors located on lactotrophs and inhibits prolactin release by inhibiting both prolactin gene expression and lactotroph mitotic activity. The TIDA system does not have a neurotransmitter-mediated reciprocal feedback circuit, as does the mesotelencephalic dopamine system (Rubin, 1987). Prolactin is able to control its own release through negative short-loop feedback via augmentation of hypothalamic TIDA turnover (Molitch, 1995). As prolactin levels rise, the amount of dopamine available to the pituitary increases. All antipsychotic medications lead to increased release and peripheral prolactin elevation by competing with dopamine at dopamine D₂ receptor binding sites located on lactotrophs.

**Antipsychotics and Prolactin: Chronic Effects**

Typical antipsychotics, such as haloperidol, have a high affinity for dopamine D₂ receptors and this leads to large and sustained increases in plasma prolactin levels in both rats (Gudelsky et al, 1987; 1989) and humans (Crawford et al, 1997; Lee et al, 1995; Meltzer & Fang,
In this regard, investigators found that 27 patients undergoing long-term treatment with typical antipsychotics continued to have sustained prolactin elevation when tested at both one and three months after the initiation of treatment (Meltzer & Fang, 1976). In addition, Crawford and coworkers (1997) found that patients undergoing treatment with haloperidol continued to maintain significantly elevated prolactin levels above the upper limit (0.6 nmol/l for males, 0.8 nmol/l for females) of normal at all time points tested over a one year period compared to olanzapine-treated patients, who showed only mild elevations during the first six weeks of treatment. No group of olanzapine-treated males experienced mean prolactin elevations above the upper limit of normal.

With respect to females, those treated with either 10 or 15 mg/day of olanzapine experienced prolactin elevations (1.3 nmol/l), which declined (0.9 nmol/l) by week 6. After this time, though medication continued, prolactin levels in male and female olanzapine-treated patients were comparable to those in the placebo group, remaining below the upper limit of normal. It is important to note, however, that this study has several limitations. The doses of haloperidol (15 ± 5 mg/day) and olanzapine (5 ± 2.5 to 15 ± 2.5 mg/day) were not comparable. This dose of haloperidol can completely saturate dopamine D₂ receptors whereas the doses of olanzapine used in this study give rise to much lower (43%-80%) D₂ occupancy (Kapur et al., 1998). Moreover, time of blood sample collection relative to sleep and medication ingestion was not controlled and prolactin levels are very sensitive to these factors.

Risperidone has also been found to lead to sustained hyperprolactinemia over the long-term course of therapy (Caracci & Ananthamoorthy, 1999). Compared to prolactin levels in 20 pre-menopausal female patients on typical antipsychotics, prolactin levels in 20 risperidone-treated female patients with schizophrenia were significantly higher (48 ng/ml versus 102 ng/ml, respectively) after 6 months of treatment (Caracci & Ananthamoorthy, 1999). Kleinberg and others (1999) pooled baseline and post-baseline prolactin data obtained from 4 separate randomized, double-blind clinical trials comparing risperidone doses ranging between 1-16 mg/day to haloperidol 10-20 mg/day in 841 patients with schizophrenia. These researchers
reported that patients receiving risperidone were found to have significantly higher serum prolactin levels than those receiving haloperidol.

Why risperidone gives rise to significantly higher plasma prolactin levels compared to olanzapine and haloperidol is not entirely clear, although evidence suggests that this may be due to the pharmacokinetic and not pharmacodynamic properties of risperidone (Bowden et al., 1992). When given at equivalent doses, risperidone yields a 3- to 4-fold higher increase in drug plasma levels compared to haloperidol. As the pituitary gland resides outside of the blood-brain barrier, it is exposed to higher concentrations of risperidone (and its active metabolite, 9-hydroxy-risperidone), leading to a significantly higher blockade of dopamine D₂ receptors. This is not observed in brain imaging studies measuring central dopamine D₂ receptor occupancy (i.e., in the striatum) since risperidone/9-hydroxy-risperidone do not effectively penetrate the blood-brain barrier (Shitij Kapur, personal communication).

Other research has found that clozapine, unlike risperidone and haloperidol, does not lead to sustained prolactin elevation (Breier et al., 1999; Meltzer et al., 1979). For example, Meltzer et al. (1979) did not find abnormally elevated prolactin levels (> 35 ng/ml for males, > 60 ng/ml for females) in patients undergoing chronic clozapine treatment (458 mg/day for females, 579 mg/day for males) 11 hours after their last dose. Breier and others (1999) also found that prolactin levels were within normal limits (< 20 ng/ml) in clozapine-treated patients (403.6 mg/day) after six weeks of continuous treatment. It is not completely understood why sustained prolactin elevation does not occur with clozapine or olanzapine but does occur with risperidone.

**Antipsychotics and Prolactin: Acute Effects**

The acute effects of antipsychotics on prolactin have been studied far less than the long-term effects. Typical antipsychotics given orally have been found to lead to rapid increases in prolactin in humans. For example, 10 drug-free individuals given a 3 mg oral dose of haloperidol showed a progressive increase in prolactin levels over a four hour period (Lee et al., 1995). In this study, the maximal peak was thought to be reached at 4 hours but no other
prolactin samples were taken after that time. Chlorpromazine administration similarly has been shown to lead to acute prolactin elevation. Meltzer (1980) studied 10 patients who had been taking 200 mg of chlorpromazine daily for two weeks over a twelve hour period after taking their full daily dose of medication. Meltzer found a 260% mean percent change in prolactin levels compared to baseline with the mean peak being reached 6 hours post-dose.

Atypical antipsychotics have been thought to spare the prolactin secreting system, however, clozapine, which exhibits relatively low D₂ receptor occupancy, has been found to lead to a steep, dose-related increase in serum prolactin concentrations in both rodents (Gudelsky et al, 1987; 1989; Meltzer et al, 1977) and humans (Lee et al, 1995; Meltzer et al, 1979). For example, investigators found that 20 mg/kg of clozapine (yielding a striatal D₂ receptor occupancy of approximately 60%), produced significant increases in plasma prolactin concentrations 30 minutes after acute injection, the magnitude of which was similar to that produced by 0.25 mg/kg of haloperidol (yielding a striatal D₂ receptor occupancy approximately above 90%) (Gudelsky et al, 1987). This effect, however, was short-lived. Prolactin levels in clozapine-treated rodents returned to near control values within 1-2 hours after acute administration, yet they remained elevated in those treated with haloperidol.

Meltzer et al. (1989) found that prolactin levels in haloperidol-treated rats were significantly elevated at 30 minutes and remained significantly above baseline at 240 minutes. Prolactin levels in clozapine-treated rats were also significantly elevated at 30 minutes yet returned to baseline at 240 minutes. This research group also used 10 mg/kg of melperone, another atypical antipsychotic, and found a prolactin elevating profile similar to that seen in clozapine-treated rats.

Other evidence suggests that clozapine, after acute administration, has a similar effect on prolactin levels in humans (Meltzer et al, 1979). Meltzer et al. (1979) studied 4 of the 13 patients who participated in their larger study and found that all patients experienced a rapid steep increase in prolactin levels which began 45-90 minutes after drug ingestion and lasted for the entire four-hour period. Lee et al. (1995), however, did not report any significant increase over
baseline after the acute administration of clozapine compared to haloperidol in 10 drug-free individuals. Nonetheless, it is important to recognize that the dose of clozapine used by Lee and others (1995) was substantially lower (50mg compared to 450mg) than that used by Meltzer and others (1979). Close inspection of the time course graph, however, shows that while haloperidol leads to a more dramatic increase in prolactin levels compared to clozapine, prolactin levels are nevertheless almost doubled between 120 to 180 minutes over baseline after acute administration of clozapine.

Risperidone has also been found to have pronounced effects on prolactin levels after its acute administration (Huang et al, 1993; Bowden et al, 1992). Huang et al. (1993) have found that the acute administration of 1mg risperidone (through either intravenous, intramuscular or oral administration) leads to a rapid increase in prolactin levels in healthy human subjects. Except for a slightly later onset of prolactin elevation after oral risperidone, the prolactin time profiles were similar among all three routes of administration. Five hours after the acute administration of risperidone, however, the mean concentration of prolactin returns to the upper limit (16 ng/ml) of normal (population) range. In rats, 0.03-1 mg/kg of risperidone (giving a striatal D2 receptor occupancy of approximately between 20-75%) has been found to be 3 to 5 times more potent than 0.125-2 mg/kg of haloperidol (giving a striatal D2 receptor occupancy of approximately between 88-100%) in stimulating prolactin levels in vivo 1 hour post-dose (Bowden et al, 1992).

These preliminary data raise doubt regarding the prolactin sparing effects of atypical antipsychotics. Prolactin levels tested at various time points appears to be increased with all antipsychotics reviewed above. Interestingly, in the Meltzer et al. (1979) study in which the effects of clozapine on prolactin were examined, they reported that “prolactin levels were not significantly different from baseline-before drug in serum samples obtained 11 hours after the last dose in 13 patients treated chronically with relatively high doses of clozapine” (p.1552). In the same study, however, they also report that prolactin levels increased dramatically after a single oral dose of medication. Therefore, while clozapine did not lead to sustained
hyperprolactinemia, it did in fact lead to an acute hyperprolactinemic state in four patients with schizophrenia. Whether acute hyperprolactinemia occurs in other patients treated chronically with clozapine and other atypical antipsychotics is not presently known. This study is an attempt to systematically explore whether 3 atypical antipsychotics given at clinically effective doses lead to acute hyperprolactinemia over the course of a single dose cycle (24 hours).

**Objective**

The objective of the following study is to measure the change in prolactin levels over a 24 hour period following a single dose of antipsychotic medication in male patients with schizophrenia.

**Hypothesis**

Based on the existing prolactin and antipsychotic medication literature,

1) All antipsychotic medications studied will give rise to some degree of prolactin elevation over baseline over the course of a single dose cycle.

**Methods**

This study was approved by the Human Subjects Review Committee of the University of Toronto. Prospective power analyses indicated that a sample of 18 male subjects with schizophrenia (6 per drug group) would sufficiently test the study hypotheses. By recruiting 6 subjects for each of 3 cells, we expected to have a sample size sufficiently large to detect an increase of at least 40% in peak prolactin concentration compared to baseline levels. With a significance level set at 0.05 and power = 0.80, 6 subjects for each of 3 cells would be required to yield a statistically significant result. Physician information sheets describing the study outline, inclusion and exclusion criteria were given to staff psychiatrists at the Centre for Addiction and Mental Health (CAMH) by the researcher (P.T). The diagnosis of schizophrenia was determined by expert clinician consensus diagnosis based on DSM-IV criteria.
Names of patients who met all study criteria and were determined, by their psychiatrist, to be capable of providing informed consent were given to the researcher. Eligible subjects were invited by the researcher, in person or by telephone, to participate in the study. Patients were included in the study if they were taking either clozapine (≥250 mg per day), risperidone (1-6 mg/day) or olanzapine (10-20 mg/day), if they had been on these medications over 4 weeks, and if the prescribed dose had remained the same for at least 1 week. Patients were excluded from participation if they had been prescribed depot antipsychotics within 6 months of the experiment, or if they were also taking antidepressant, antiparkinsonian or other antipsychotic medication. Other concomitant medications were permitted. Written informed consent was obtained from each subject after the study had been explained. Eighteen male subjects with schizophrenia, mean age of 32 ± 8 years, participated in this research study. All subjects were recruited from the Centre for Addiction and Mental Health, Clarke and Queen Street Sites, Toronto.

Procedure

On the day before the study, subjects were asked to fast overnight and not take their evening antipsychotic medication. On the day of the study, subjects arrived at the Positron Emission Tomography (PET) Centre located at the Centre for Addiction and Mental Health, Clarke Site at approximately 8:00am. An indwelling intravenous catheter was inserted into the subject’s forearm vein for repeated blood sampling (10cc/sample). After the intravenous catheter had been in place for 30 minutes, two separate baseline blood samples were obtained at 15 minutes apart during the fasting state. After the second baseline blood sample was obtained, subjects were instructed to take a single oral dose of their currently prescribed antipsychotic medication with water in front of the study investigator. Blood samples were obtained and vital signs were measured every 60 minutes over an 8 hour period. Subjects remained in the examination room either sitting or in bed throughout the experiment. Subjects were provided with a standard breakfast and lunch at 9:30am and 12:30pm, respectively. Once the blood sample for the 8th hour was obtained, the intravenous catheter was removed. Subjects returned to
their place of residence for the remainder of the evening and were asked to refrain from taking any antipsychotic medication until the following day and to fast overnight.

On the second day of the study before breakfast, subjects returned to the PET Centre at approximately 9:00am so that a final blood sample could be obtained via veinipuncture 24 hours after their last dose of antipsychotic medication. Only after this blood sample did they take their daily medication. Subjects were also given the option to return and repeat the entire procedure on a separate occasion, but without taking their antipsychotic medication on the day of the study in order to act as their own drug-free controls. Five of the 18 subjects agreed. Blood was centrifuged in glass tubes and plasma was stored in plastic tubes at -80°C until prolactin assays were completed. Plasma prolactin levels were determined using Microparticle Enzyme Immunoassay (MEIA) technology with a minimum detectable limit of 0.6 ng/ml and both an intra-subject and inter-subject coefficient of variance of 3.6% and 4.5%, respectively (IMX™). The upper limit of normal for plasma prolactin is 20 ng/ml for males during the waking state based on current clinical guidelines at the CAMH.

**Data Analysis**

Statistical analyses proceeded in five phases. First, descriptive statistics were calculated for all 18 subjects as a single group and then separately for each drug group. Second, an ANOVA was computed to compare absolute plasma PRL levels at baseline between drug groups. Third, a repeated measures ANOVA with Group as the between-subjects factor and Time as the within-subjects factor was computed. Linear, quadratic and cubic models were analyzed to determine which model best fit the data. Fourth, post-hoc t-tests were computed to examine the time to reach mean PRL peak between each drug group, and to determine whether the mean PRL peak was significantly elevated above each respective drug groups baseline PRL plasma level. Finally, an ANOVA was also computed to compare absolute plasma PRL levels at 24 hours from baseline within each drug group. A p value of \( \leq 0.01 \) was selected to control for multiple comparisons.
Results

Eighteen male patients with schizophrenia entered the study (Table 1). Median doses for each drug group were as follows: olanzapine 20 mg (range 10-20 mg), risperidone 3 mg (range 1-3 mg), and clozapine 300 mg (300-500 mg). One clozapine subject who was currently being prescribed 500 mg of clozapine per day refused to take his full daily dose yet consented to take 400 mg. Equivalent doses among the 3 groups could not be achieved because of the small sample.

An ANOVA revealed a statistically significant difference in mean baseline plasma PRL levels between risperidone (27 ± 14 ng/ml), olanzapine (9 ± 5 ng/ml) and clozapine (9 ± 5 ng/ml) (F = 11.43; df = 2,15; p < 0.01).

Using absolute plasma PRL values, a repeated measures ANOVA was conducted revealing a significant Group effect [F (2,15) = 27.54; p < 0.01], a significant Time effect [F (9,135) = 6.37; p < 0.01] and a significant Group × Time interaction [F (18,135) = 2.44; p < 0.01] (Table 2a).

A linear trend model revealed a significant Group effect [F (2,15) = 27.54; p < 0.01], but not a significant Time effect [F (9,135) = 0.32; p > 0.05] or Group × Time interaction [F (18,135) = 2.71; p > 0.05] (Table 2b).

Using a quadratic trend model, the repeated measures ANOVA revealed a significant Group effect [F (2,15) = 27.54; p < 0.01], a significant Time effect [F (9,135) = 25.14; p < 0.01], but not a significant Group × Time interaction [F (18,135) = 1.52; p > 0.05] (Table 2c).

Using a cubic model, the repeated measures ANOVA revealed a significant Group effect [F (2,15) = 27.54; p < 0.01], a significant Time effect [F (9,135) = 11.62; p < 0.01], and a significant Group × Time interaction [F (18,135) = 4.90; p < 0.01] (Table 2d).

Post hoc tests revealed the following. Time to reach peak PRL levels were significantly different among groups [F (2,15) = 17.15 p < 0.01]. Risperidone mean peak PRL levels occurred significantly earlier (2 hrs) (Figure 2a) compared to olanzapine (5 hrs) (Figure 2c) [p < 0.01], but not clozapine (3 hrs) (Figure 2b) [p > 0.05]. This is in agreement with the time of peak
concentration in plasma from initial dose ($t_{\text{max}}$) values for each drug: clozapine 1-3 hours; risperidone 0.8-1.5 hours; and olanzapine 5 hours.

CLZ patients showed a mean increase from $9 \pm 5$ to $24 \pm 8$ ng/ml [$p<0.01$], OLN patients showed a mean increase from $9 \pm 5$ to $18 \pm 10$ ng/ml [$p<0.01$] and RIS patients showed a mean increase from $27 \pm 14$ to $55 \pm 14$ ng/ml [$p=0.05$].

Finally, there was also a statistically significant difference in mean plasma PRL levels obtained at 24 hours compared to each drug group's mean baseline between risperidone ($31 \pm 13$ ng/ml), olanzapine ($14 \pm 8$ ng/ml) and clozapine ($8 \pm 5$ ng/ml) ($F(2,15) = 9.47; p < 0.01$).

Mean olanzapine PRL levels remained significantly elevated above baseline at 24 hours [$p < 0.05$]. The data revealed that 3 subjects had significantly higher PRL levels at 24 hours compared to their baseline values (24 ng/ml compared to 10 ng/ml for S1, 22 ng/ml compared to 15 ng/ml for S2, and 14 ng/ml compared to 9 ng/ml for S3). All 3 subjects denied taking their morning medication prior to the 24 hour PRL sample. To confirm this, olanzapine plasma samples obtained at 5 and 24 hours were analyzed to determine whether plasma levels obtained at 24 hours were similar than those obtained at 5 hours ($t_{\text{max}}$ of olanzapine). Laboratory drug plasma levels confirmed each subject's compliance with the study protocol (188 nmol/L at 5 hours compared to 95 nmol/L at 24 hours for S1, 85 nmol/L at 5 hours compared to 36 nmol/L at 24 hours for S2, and 76 nmol/L at 5 hours compared to 40 nmol/L at 24 hours for S3).

Control Data

Within subject control data were obtained for five participants (clozapine = 1, risperidone = 2, olanzapine = 2) to determine whether any predicted changes in prolactin levels were not simply the result of the natural circadian rhythm of prolactin secretion. Figures 3a-e demonstrate that there is a significant difference in prolactin levels over the course of 24 hours between each subject's on-drug and off-drug days that cannot be explained by normal prolactin fluctuations. Each subject demonstrated only minor, if any, plasma prolactin fluctuations.
during the control experiment. Since few subjects agreed to take part in the control phase of the study, statistical comparisons among groups on the on- and off-drug days were precluded.

Discussion

These results help in clarifying our thinking about the meaning of atypicality when describing antipsychotics. Lack of prolactin elevation is considered a defining feature of atypicality. These data, however, indicate that all of the atypical antipsychotics tested in this study give rise to acute PRL elevation, even after chronic dosing. Studies which have examined the prolactin profile of atypical antipsychotics have obtained prolactin levels 12-24 hours after the last oral dose (Meltzer et al., 1979; Breier et al., 1999). Our study, however, demonstrates that the prolactin increases observed at 3-6 hours may return to baseline by 12-24 hours, giving the appearance that there is no effect on prolactin levels. Therefore, the differences among typical and atypical antipsychotics may not be categorical (i.e. PRL elevation vs. none) but quantitative, in that typical antipsychotics gives rise to sustained prolactin elevations, whereas clozapine, risperidone and olanzapine give rise to transient prolactin increases which vary between these agents. Risperidone plasma prolactin levels show the 'fastest' transience, clozapine is 'moderately' transient and olanzapine shows the 'slowest' transience. Should these results be reproducible, the typical/atypical typology of the prolactin/antipsychotic relationship must be redefined.

There are several important issues which limit interpretation of these data. First, 3 risperidone-treated subjects, each of whom was defined as having hyperprolactinemia (plasma PRL level > 20 ng/ml) at baseline, had minimal change in plasma PRL levels after ingestion of their antipsychotic medication. Subjects with hyperprolactinemia are known to have blunted PRL responses secondary to a pharmacological challenge (Molitch & Reichlin, 1994). While the physiological basis for this is not well understood, it has been suggested that there are two different pools of prolactin in the pituitary, one which turns over rapidly while the other turns over slowly (Molitch & Reichlin, 1994). Hyperprolactinemia may be associated with a small
hormone pool that is turning over rapidly (Molitch & Reichlin, 1994). Therefore, prolactin monitoring in future studies should exclude subjects with hyperprolactinemia.

Second, the physiology of the prolactin secreting system poses natural limits to the usefulness of prolactin monitoring. Movin-Osswald et al. (1995) have suggested that the kinetics of prolactin response are determined by the direct influence of the antipsychotic on prolactin, the prolactin pool size, as well as the formation and elimination rate of prolactin from plasma. Thus, the ability of an antipsychotic to stimulate prolactin release may be larger than the ability of prolactin to respond to it due to the amount of readily releasable prolactin, and thus restricted by size of the prolactin pool (Movin-Osswald et al., 1995). This suggests that each of the subjects recruited into this study may have had decreased prolactin response as a result of long-term antipsychotic treatment. Therefore, a separate study including antipsychotic-naïve patients with schizophrenia and/or normal controls would be beneficial in that it would provide prolactin data in individuals with a relatively unperturbed prolactin secreting system.

The control data demonstrate that there is a clear difference in extent of prolactin elevation when comparing each individual subject’s data between on- and off-drug days. While some subjects did demonstrate prolactin elevations during the off-drug day, it occurred within 1 hour of meal consumption. Some evidence suggests that prolactin levels may increase by 50% to 100% within 30 minutes of meals (Quigley et al., 1981), possibly due to the increase in amino acids that are generated from the protein component of the meal (Carlson et al., 1983, 1989).

What is difficult to explain, however, is why the olanzapine-treated group had significantly higher plasma prolactin levels at 24 hours compared to their baseline values on both on- and off-drug days. The relatively high prolactin level may have been due to the fact that the time of blood sampling coincided with the early morning peak characteristic of prolactin secretion. However, each subject’s baseline levels were taken at approximately the same time, 24 hours after their last dose of oral medication. Moreover, no subject reported sleep disturbances the night of the study and each awoke at approximately the same time on both mornings. This is unlikely to be an olanzapine effect because we did not observe this for 3 out of
the 6 olanzapine-treated subjects. On the other hand, no one in the other drug groups showed this effect.

One other possible explanation relates to the half-life of olanzapine. It is well known that there are inter-individual differences in elimination half-life of almost all drugs. Since olanzapine has a longer half-life compared to clozapine and risperidone (Jibson & Tandon, 1998), and since drugs with longer half-lives may give prolactin peaks with a slower decline (Movin-Osswald et al., 1995), had a sample been collected later on in the day (i.e., 28-30 hours), the prolactin levels may have returned to baseline. This, however, does not account for the elevated prolactin levels obtained at 24 hours compared to baseline during the off-drug day for the 2 olanzapine subjects.

Why certain antipsychotics give rise to sustained prolactin levels whereas others give rise to only transient increases is not well understood. Seeman and Tallerico theorize that antipsychotic medications differ in their duration of binding to dopamine D₂ receptors (1998, 1999). This is based on the $k_{on}$ $k_{off}$ theory of drug/receptor interactions (Limbird, 1986). The rate constant $k_{on}$ determines how fast a drug binds to a receptor, given a certain synaptic concentration. Conversely, the rate constant $k_{off}$ determines how fast a drug comes off a receptor (Limbird, 1986). These two factors are theorized to predict the interactions between an antipsychotic drug and brain receptors. Clozapine and quetiapine, both believed to bind "loosely" to dopamine D₂ receptors are rapidly displaced from dopamine D₂ receptors by endogenous dopamine (Seeman and Tallerico, 1998; 1999).

For example, $[^3]$H clozapine prebound to D₂ receptors is displaced by endogenous dopamine approximately 100 times faster than $[^3]$H haloperidol or $[^3]$H olanzapine (Seeman and Tallerico, 1999). This theory has been used to explain why antipsychotics vary in their dopamine D₂ receptor occupancies. It also seems to explain rapidity of clinical relapse upon abrupt clozapine or quetiapine discontinuation (Parsa et al., 1993, Thyrum et al., 1997). This has been observed to differ among different therapeutic compounds (Gilbert et al., 1995). It may also explain why different antipsychotics have different prolactin elevating effects. According to
this theory and in vitro studies, clozapine binds loosely to the dopamine D₂ receptor and should induce a short-lived prolactin elevation. The same would be true theoretically for quetiapine. Olanzapine, which binds "moderately loosely" to the dopamine D₂ receptor should produce longer lasting prolactin elevations. Haloperidol and risperidone and its metabolites bind "tightly" to the dopamine D₂ receptor. Prolactin elevations with these drugs should be both higher and more prolonged.

These data, however, only demonstrate a minor relationship between the prolactin elevating profiles of antipsychotics and their respective \( k_{\text{off}} \) values. Mean plasma prolactin levels in patients treated with clozapine, an antipsychotic which binds "loosely" to the dopamine D₂ receptor, were normal at baseline, peaked early and returned to baseline by 24 hours. Mean plasma prolactin levels in patients treated with olanzapine, which binds "moderately loosely" to the dopamine D₂ receptor, were also normal at baseline, peaked later than clozapine, but remained elevated above baseline at 24 hours. For risperidone, a drug which binds "tightly" to the dopamine D₂ receptor, mean plasma prolactin levels were abnormally elevated at baseline, peaked early and returned to baseline at 24 hours. Therefore, while the \( k_{\text{off}} \) value of an antipsychotic may predict whether a drug will lead to sustained hyperprolactinemia measured at baseline, it cannot be used to predict the pattern of acute prolactin elevation.

**Further Considerations**

Aside from the aforementioned issues, this study has other limitations. Sample size is an important issue for generalizability of findings. It would have been ideal to have recruited more subjects into each drug group but it proved to be very difficult to find patients who met all inclusion criteria and who were willing to devote a full day and a half or, in some cases, two days to the project.

The study was done on an all male sample so the findings may not apply to women. Prolactin response to antipsychotic medication is more robust in women. Women were excluded
from the study in order to make the sample more homogeneous. A comparable sample of women would have been ideal, however, in the pre-screening phase of this study we found that many female patients being treated with antipsychotics also receive concomitant antidepressant treatment which excluded them from this study.

Another problem was that drug dose was not controlled. The subjects within each drug group were on different doses of the same antipsychotic. The variable responses of prolactin within each drug group could, in part, be explained by variable dose-related increases in plasma prolactin levels. We did not study patients on antipsychotic medications other than olanzapine, risperidone and clozapine. It would have been ideal to include other antipsychotics. As previously mentioned, however, we found very few patients receiving oral dose monotherapy with antipsychotics other than the ones selected.

**Future Directions and Conclusions**

In conclusion, we have shown that each of the tested atypical antipsychotics in this study lead to an acute rise in prolactin levels. While it is true that most atypicals, excluding risperidone, do not lead to sustained hyperprolactinemia, they do lead to a short-lived acute hyperprolactinemic state, the clinical significance of which is not known. Therefore, the original assumption that antipsychotics classified as atypical spare the prolactin secreting system in humans is not altogether accurate. Our data do support the view that the prolactin/antipsychotic-type is more complex than previously thought. All antipsychotics produce prolactin elevations but some drugs cause only transient elevations which may have no clinical consequence. This, however, remains to be determined.
References


65. Seeman, P.; and Tallerico, T. Antipsychotic drugs which elicit little or no parkinsonism bind more loosely than dopamine to brain dopamine D2 receptors, yet they occupy high levels of these receptors. *Mol Psychiatry, 3*:123-34, 1998.


Table 1. Characteristics of Male Subjects

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### Table 2b: ANOVA Summary: % Difference From Baseline (Linear Model)

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Figure 1. Normal Diurnal Variation of Prolactin Secretion Over a 24-Hour Period in Healthy Controls (Molitch, 1995).
Figure 2a. Mean (SE) plasma PRL levels in 6 male subjects treated with risperidone (Median dose 3 mg/day). Plasma PRL levels peaked by 2 hours and returned to baseline by 24 hours.
Figure 2b. Mean (SE) plasma PRL levels in 6 male subjects treated with clozapine (Median dose 300 mg/day). Plasma PRL levels peaked by 2 hours and returned to baseline by 24 hours.
Figure 2c. Mean (SE) plasma PRL levels in 6 male subjects treated with olanzapine (Median dose 20 mg/day). Plasma PRL levels peaked by 5 hours and did not return to baseline by 24 hours ($p < 0.05$).
Figure 3a. Percentage change in plasma PRL levels from baseline in 1 male subject both on and off 300 mg/day of clozapine.
Figure 3b. Percentage change in plasma PRL levels from baseline in 1 male subject both on and off 12.5 mg/day of olanzapine.
**Figure 3c.** Percentage change in plasma PRL levels from baseline in 1 male subject both on and off 20 mg/day of olanzapine.
Figure 3d. Percentage change in plasma PRL levels from baseline in 1 male subject both on and off 1 mg/day of risperidone.
Figure 3e. Percentage change in plasma PRL levels from baseline in 1 male subject both on and off 3 mg/day of risperidone.