CLOZAPINE INCREASES RAT SERUM PROLACTIN LEVELS
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Summary

Clozapine differs from other anti-psychotic drugs in that it produces little or no extrapyramidal side effects. The effects of clozapine on rat brain dopamine differ markedly from those of the neuroleptic drugs. The neuroleptics increase rat serum prolactin levels which has been attributed to their dopamine receptor blocking properties. We found that clozapine markedly increased serum prolactin levels in male rats when injected intraperitoneally in doses of 5, 10, 50 and 100 mg/kg. Serum prolactin levels after 5 mg/kg clozapine were significantly less than in rats given 10, 50 and 100 mg/kg which did not significantly differ from each other. Serum prolactin after 10 mg/kg clozapine was significantly greater than after chlorpromazine, 5 mg/kg and haloperidol, 0.5 mg/kg. The increases in serum prolactin are attributed to clozapine's ability to produce dopamine blockade or to inhibit nerve impulse-dopamine release, or both. The capacity of clozapine to affect brain serotonin and norepinephrine metabolism and its strong anti-cholinergic properties are probably not involved in its ability to increase serum prolactin.

Clozapine is an effective anti-psychotic agent whose mechanism of action is uncertain. Unlike the neuroleptic anti-psychotic drugs such as chlorpromazine and haloperidol, it produces little or no extrapyramidal side effects (EPS) (1-3). The major effect of the neuroleptics is believed to be dopamine receptor blockade which leads to feedback activation of increased dopamine turnover, as indicated by increased brain homovanilliac acid (HVA) (4,5). Clozapine also has definite effects on brain dopamine metabolism but there is conflicting evidence, which will be discussed subsequently, as to whether it is a dopamine receptor blocker (6-10).

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Recently, it has become possible to study the effects of chemical agents on tuberoinfundibular dopamine neurons and pituitary dopamine receptors by measuring their effects on serum prolactin. Drugs which promote dopaminergic activity by increasing synthesis (L-Dopa), by agonist properties (apomorphine), or by inhibiting dopamine catabolism (pargyline) decrease serum prolactin; drugs which decrease dopaminergic activity by inhibiting synthesis (alpha-methylparatyrosine) or blocking dopamine receptors (chlorpromazine) increase serum prolactin (11-13). Thus, neuroleptic anti-psychotic drugs, but not chemically similar compounds which lack anti-psychotic effects, raise serum prolactin (12-14). We therefore decided to study the effects of clozapine on rat serum prolactin to determine if it might affect this dopamine system.

**Methods**

Male 150-175 gm Sprague-Dawley rats obtained from Sprague-Dawley, Inc., Madison, Wisconsin were used in this study. All rats were fed Purina rat chow and water ad libitum. The rats were kept in a room at 24°C with a 12 hr light-dark cycle. All drugs were injected i.p. There were four rats per group. Rats were killed by decapitation 30 minutes after drug administration. Serum was collected and assayed for prolactin by a modification of a double antibody radioimmunoassay method originally developed for human prolactin (15). The plasma concentrations are expressed as ng/ml of NIAMDD rat-PRL-RP-1. Clozapine was a gift of Sandoz, Ltd. It was dissolved in dilute acetic acid. Haloperidol was a gift of McNeil Laboratories. Chlorpromazine HCl was a gift of Smith, Kline and French, Inc.

The mean levels in drug-treated groups were compared with each other and with control levels by means of the Student's t-test. The criterion for statistical significance was p<0.05.

**Results**

Clozapine markedly increased rat serum prolactin levels at doses of 5, 10, 50 and 100 mg/kg i.p. (Table 1). Serum prolactin levels in rats given 5 mg/kg clozapine were significantly less than the levels in rats after 10, 50 and 100 mg/kg which did not significantly differ from each other. Serum prolactin after 10 mg/kg clozapine was significantly greater than after chlorpromazine, 5 mg/kg.

### TABLE

| Treatment* | Dose (mg/kg) | Plasma Prolactin+ (ng/ml) | p<^\text{g} | *
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<tr>
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<tbody>
<tr>
<td>Vehicle</td>
<td>---</td>
<td>7.1±4.6</td>
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<td>Clozapine</td>
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<td>57.6±30.2</td>
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<tr>
<td>Chlorpromazine</td>
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<td>Haloperidol</td>
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<td>36.7±13.2</td>
<td>0.025</td>
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* Groups of 4 rats were injected i.p. 30 min prior to decapitation  
+ Mean±S.D.  
^ Significance of difference from saline group, Student t-test.
and haloperidol, 0.5 mg/kg. These agents produced significant increases in prolactin that were not significantly different from those produced by clozapine, 5 mg/kg.

**Discussion**

Clozapine increased serum prolactin levels at all doses studied. Before considering the implications of this for the effect of clozapine on brain dopamine metabolism, it is important to consider other effects of clozapine which might mediate this increase.

Anti-cholinergic agents such as atropine have been shown to decrease or have no significant effect on serum prolactin in female rats (16-18) or have no significant effect on serum prolactin in male rats (Meltzer and Fang, unpublished observations). Although clozapine has strong anti-cholinergic properties (19,20), this property should not contribute to the increase in serum prolactin.

Clozapine has potent anti-adrenergic properties (6,21). However, alpha- and beta-adrenergic blockers increase serum prolactin only at very high doses (18), so it is unlikely that the adrenergic blocking properties of clozapine mediate the large increases in serum prolactin seen after low doses of clozapine.

Clozapine increases rat brain serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) levels without changing 5-HT turnover (6,9). Drugs which increase brain 5-HT increase rat serum prolactin (22). However, the effects of clozapine on brain 5-HT are not yet manifest at doses as high as 20 mg/kg whereas the increase in serum prolactin is already marked at 5 mg/kg.

The increase in serum prolactin could be due to dopamine receptor blockade as has been proposed for chlorpromazine and other neuroleptics (12-14). The evidence for clozapine as a dopamine blocker is mixed: increased HVA levels, although only at relatively high doses, perhaps due to the anti-cholinergic effects of clozapine (7-10); and inhibition of the putative dopamine receptor, a dopamine-sensitive adenyl cyclase (23-25). On the other hand, the finding that all neuroleptics except clozapine augmented the disappearance of 14C-dopamine formed from 14C-tyrosine in mouse brain argues against clozapine having dopamine receptor blocking properties (26). Bartholini has proposed that if clozapine does produce receptor blockade, it is of the type which is surmountable by increased dopamine release, which, if so, would be especially true at low doses (6). Thus, low doses of clozapine should produce little or no effect on serum prolactin whereas we found a significant increase in serum prolactin even at low doses which do not increase brain HVA.

A number of the reported effects of clozapine are consistent with clozapine as an inhibitor of dopamine release which, if it occurred in the hypothalamus, would also lead to increased serum prolactin. Clozapine, in doses of 1.25-5 mg/kg, produced a nearly significant decrease in the disappearance of 14C-dopamine formed from 14C-tyrosine (23). Inhibition of release could contribute to the increased brain dopamine (8,9) produced by clozapine, although not all investigators have found significant increases (6,10). Clozapine has an additive effect with low doses of prochlorperazine in the production of catalepsy (6). Decreased dopamine release would synergize with receptor blockade to promote catalepsy. Finally, blockade of release could contribute to the suprasensitivity to the development of EPS produced by chlorpromazine when the latter drug is given after discontinuation of clozapine (2). Suprasensitivity would develop from the prior relative absence of dopamine at the dopamine receptor.
Interference with nerve impulse flow has been proposed as the basis of the anti-psychotic action of all neuroleptics (27,28). Seeman and Lee have reported that all types of anti-psychotic agents, including clozapine, block the electrically-stimulated release of 3H-dopamine from rat caudate slices and that the impulse-blocking potencies correlate significantly with the anti-psychotic potencies of the neuroleptics (29). Conceivably, inhibition of impulse-mediated dopamine release might contribute to the serum prolactin-stimulating properties of neuroleptic anti-psychotics as well as their anti-psychotic effects.

Further studies will be needed to determine the relative importance of dopamine receptor blockade and inhibition of dopamine release to the increase in serum prolactin and anti-psychotic effects of clozapine and neuroleptic anti-psychotic drugs.

References