Evidence for a pathological reduction in brain dopamine metabolism in idiopathic hyperprolactinemia

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Abstract. The role of brain catecholamine activity in the neuroendocrine regulation of the dopamine-PRL system in idiopathic hyperprolactinemia was investigated by high-performance liquid chromatography with electrochemical detector. We measured urinary dopamine, noradrenaline, epinephrine, vanillylmandelic acid, homovanillic acid, 3,4-dihydroxyphenyleacetic acid and total 3-methoxy-4-hydroxyphenylglycol levels in 12 women with idiopathic hyperprolactinemia before and during either peripheral dopa-decarboxylase blockade, by carbidopa, or dopamine β-hydroxylase blockade, by disulfiram. Homovanillic acid and 3,4-dihydroxyphenyleacetic acid concentrations were significantly lower (p<0.001 and p<0.005, respectively) in patients with idiopathic hyperprolactinemia compared with those in 12 control subjects in the early follicular phase, whereas they were similar to those in the control subjects in the pre-ovulatory phase. Dopamine, norepinephrine, epinephrine, vanillylmandelic acid and 3-methoxy-4-hydroxyphenylglycol concentrations were similar to those of the control subjects in both phases of the cycle. During carbidopa administration the levels of all urinary catecholamines and metabolites were unchanged, except that of dopamine which dropped remarkably (p<0.001). During disulfiram administration dopamine, homovanillic acid and 3,4-dihydroxyphenyleacetic acid concentrations increased (p<0.05, p<0.001 and p<0.005, respectively) and those of norepinephrine, vanillylmandelic acid and 3-methoxy-4-hydroxyphenylglycol decreased (p<0.05, p<0.001 and p<0.005, respectively), whereas epinephrine levels remained unaltered. These data support the existence of a quantitatively reduced brain dopamine activity in idiopathic hyperprolactinemia.

Via the median eminence and hypophysial portal blood, hypothalamic dopamine is the primary physiological inhibitor of pituitary PRL secretion in humans (1). Hyperprolactinemia associated with idiopathic hyperprolactinemia, with all known causes excluded and with a normal computed tomography (CT) scan, the diagnosis of idiopathic hyperprolactinemia is made occasionally (2). It is not known, whether idiopathic hyperprolactinemia and prolactinoma are two distinct entities or two subsequent phases of the same disease (2). The etiology of both disorders, if two, remains largely unresolved. Various PRL stimulation and suppression tests by dopamine-agonists and -antagonists, dopamine receptor blockers, and dopamine synthesis inhibitors, have been performed (3,4). These indirect studies, based on exogenous administration of pharmacological doses of dopamine-mimicking drugs show questionable and contradictory results not allowing conclusions on the pathophysiological importance of such observations. However, it has been hypothesized that PRL hypersecretion may be the result of a defect in the central nervous system (CNS)-dopamine neurotransmission (5,6), and, particularly, of a supposed reduction in dopamine release (1).
To ascertain the existence of these defects in idiopathic hyperprolactinemia, we directly investigated central dopamine activity and studied, in details, urinary catecholamines and their preferentially peripheral and CNS-derived metabolites before and during either peripheral dopa-decarboxylase blockade, by carbidopa, or dopamine β-hydroxylase blockade, by disulfiram, in a group of patients with idiopathic hyperprolactinemia. We performed a direct urinary assay of dopamine, nor-epinephrine, epinephrine, unconjugated catecholamines of predominantly peripheral origin (7-9), vanillylmandelic acid (VMA) (3-methoxy-4-hydroxymandelic acid), a metabolite of predominantly peripheral noradrenergic activity (10,11), homovanillic acid (HVA) (3-methoxy-4-hydroxyphenylacetic acid), and 3,4-dihydroxyphenylacetic acid (DOPAC), the major metabolites of CNS dopaminergic activity (7,8), and total (sulphate and glucuronide) 3-methoxy-4-hydroxyphenylglycol (MHPG), the major metabolite of CNS noradrenergic activity (10,11).

Patients and Methods

Twelve women aged 22-46 years, with clinical and laboratory evidence of hyperprolactinemia, who had been followed for at least one year, were studied. The women, with all known causes of hyperprolactinemia excluded, had normal radiological findings (high resolution computed tomography). All suffered from oligo/amenorrhea and infertility with or without galactorrhea. Most of them had also slightly increased body weights. All had constantly sustained hyperprolactinemia (between 50 and 150 μg/l in 10 blood samples drawn every 20 min for 3 h from 08.00 to 11.00 h), had normal-low serum gonadotropic concentrations, as well as normal-low levels of gonadal sex steroids. The women were diagnosed as having idiopathic hyperprolactinemia and their clinical findings are summarized in Table 1. For comparison, 12 age-, height- and weight-matched normally cycling women served as controls.

**Experimental design**

After informed consent, the subjects were admitted to our Clinical Research Centre as out-patients, instructed to continue their usual life-styles to avoid stress-induced interferences on catecholamine metabolism, and warned to avoid any alcohol-containing preparation during and several weeks after the discontinuation of the study. None had received any medication for at least 6 months before the study. Oligomenorrheic women were investigated during the early follicular phase (day 2-13 of the cycle) and amenorrheic women at any time for 12 days according to the following manner: On day 1 a 24-h urine collection was obtained from each subject to avoid interferences owing to circadian variations in catecholamine levels and their metabolites; in addition, 7 blood samples were drawn every 20 min for 3 h from 08.00 to 11.00 h.

### Table 1.

Clinical parameters in women with idiopathic hyperprolactinemia.

<table>
<thead>
<tr>
<th>Patient (No.)</th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Body weight (kg)</th>
<th>BMI</th>
<th>Menarche</th>
<th>Menses</th>
<th>Galactorrhea</th>
<th>Basal PRL (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>1.64</td>
<td>48</td>
<td>17.8</td>
<td>12</td>
<td>O</td>
<td>+</td>
<td>80.0 (3.1) b</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>1.62</td>
<td>67</td>
<td>25.5</td>
<td>11</td>
<td>O</td>
<td>+</td>
<td>82.7 (3.9)</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>1.70</td>
<td>67</td>
<td>23.3</td>
<td>12</td>
<td>A d</td>
<td>-</td>
<td>171.1 (19.1)</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>1.63</td>
<td>90</td>
<td>33.9</td>
<td>11</td>
<td>O</td>
<td>-</td>
<td>55.3 (4.6)</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>1.62</td>
<td>60</td>
<td>22.9</td>
<td>11</td>
<td>O</td>
<td>-</td>
<td>132.2 (11.5)</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>1.65</td>
<td>75</td>
<td>27.5</td>
<td>12</td>
<td>O</td>
<td>+</td>
<td>99.7 (3.6)</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>1.55</td>
<td>74</td>
<td>30.8</td>
<td>13</td>
<td>O</td>
<td>+</td>
<td>55.4 (2.2)</td>
</tr>
<tr>
<td>8</td>
<td>26</td>
<td>1.72</td>
<td>64</td>
<td>21.6</td>
<td>13</td>
<td>A</td>
<td>-</td>
<td>150.9 (7.8)</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>1.65</td>
<td>52</td>
<td>19.1</td>
<td>11</td>
<td>A</td>
<td>+</td>
<td>151.1 (8.6)</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
<td>1.56</td>
<td>47</td>
<td>19.3</td>
<td>12</td>
<td>O</td>
<td>+</td>
<td>59.1 (2.8)</td>
</tr>
<tr>
<td>11</td>
<td>43</td>
<td>1.57</td>
<td>55</td>
<td>22.3</td>
<td>12</td>
<td>O</td>
<td>+</td>
<td>79.6 (9.5)</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>1.56</td>
<td>60</td>
<td>24.7</td>
<td>12</td>
<td>O</td>
<td>+</td>
<td>61.7 (4.9)</td>
</tr>
</tbody>
</table>

Mean

SEM

BMI, body mass index (body weight (kg)/height (m)^2); b Mean (SEM) in 10 blood samples; O, oligomenorrhea; A, amenorrhea

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were drawn every 10 min for 1 h from 08.00 to 09.00 h from an indwelling catheter and then pooled to avoid interferences of an hourly hormonal pulsatility. At 20.00 h of the same day each subject began to take 25 mg/6 h of carbi-dopa (C-dopa) (Merck Sharp Dohme, Rome, Italy), a specific peripheral dopa-decarboxylase inhibitor (12), for 3 days (total dose, 300 mg). Additional 24-h urine and blood samples were then obtained on day 4. Basal 24-h urine and blood samples were again collected on day 8. At 20.00 h each subject began to take 400 mg/12 h of disulfiram (Crinos, Como, Italy), a specific dopamine β-hydroxylase inhibitor (13,14), for 4 days (total dose, 3200 mg). The fourth and last 24-h urine collection and blood sampling were then obtained on day 12.

No adverse reactions were observed during C-dopa administration, whereas gastrointestinal upsets, headache, drowsiness and fatigue were observed in most of the subjects during disulfiram administration. In one subject an unpleasant taste and allergic dermatitis was also noted, but in none the symptoms were so severe that the study had to be stopped.

A 24-h urine collection and 7 blood samples were obtained from control subjects as well as in the patients with idiopathic hyperprolactinemia during both the early follicular (day 3 of the cycle) and the pre-ovulatory phases (day -1 with respect to midcycle LH surge), as previously reported (15).

Urinary determinations were for creatinine, dopamine, norepinephrine, epinephrine, VMA, HVA, DOPAC and total MHPG. Blood determinations were for LH, FSH, PRL, GH, TSH, E2, testosterone and progesterone. Plasma samples were separated and stored frozen at −20°C until assayed. Urine was acidified by HCl (0.1 mol/l of urine) during collection, and the volume of each 24-h sample was immediately measured, and aliquots taken and frozen at −70°C until analysed.

**Assays**

Peptide hormones were measured by double-antibody RIA techniques using commercial kits: plasma LH and FSH, IU/l, 2nd International Reference Preparation (IRP) of HMG (Biodata, Rome, Italy); plasma PRL, µg/l, 1 µg equivalent to 1 µg NIH-F1 (Biodata); plasma GH, µg/l, 1 µg equivalent to 2 mIU 1st IRP, MCR 66/217.

**Table 2.**

Plasma levels of LH, FSH, PRL, GH, TSH, progesterone, testosterone and estradiol (E2) in patients with idiopathic hyperprolactinemia before and during (C-dopa) and disulfiram (TTD) administration. Data from control subjects are reported for comparison.

<table>
<thead>
<tr>
<th></th>
<th>Plasma Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH (IU/l)</td>
</tr>
<tr>
<td>Patients with idiopathic hyperprolactinemia (N=12)</td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td>with C-dopa</td>
</tr>
<tr>
<td>Control subjects (N=12)</td>
<td>EFP</td>
</tr>
<tr>
<td></td>
<td>POP</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM. EFP, early follicular phase; POP, pre-ovulatory phase.

*p<0.05, **p<0.005, ***p<0.001 compared with respective baseline value in patients with idiopathic hyperprolactinemia and EFP value in control subjects.

†p<0.05, ††p<0.005, †††p<0.001 compared with EFP value in control subjects.

*p<0.05, **p<0.005, †††p<0.001 compared with POP value in control subjects.
(Sclavo, Siena, Italy); plasma TSH, mIU/l, 1st IRP, WHO 68/38 (Diagnostic Products Corporation, Los Angeles, CA). The sensitivity of the method and percentage intra-assay (inter-assay) variability were 1.5 IU/l and 5 (8), 1.5 IU/l and 4.5 (6.5), 1.5 µg/l and 4 (7), 0.3 µg/l and 6 (11), and 0.2 mIU/l and 6.5 (12.5) for LH, FSH, PRL, GH, and TSH, respectively. All samples were assayed in duplicate in a single assay. E2, progesterone and testosterone, were measured by RIA techniques, as previously reported (16). The sensitivity of the method and percentage intra-assay (inter-assay) variability were: 15 pg/tube and 11.0 (16.2), 2.6 pg/tube and 5.5 (6.4), and 15 pg/tube and 5.0 (9.8) for progesterone, E2 and testosterone, respectively.

Urinary creatinine was measured by AutoAnalyzer (Smac, Technicon, New York, NY). All the urinary catecholamines and metabolites were extracted using solid-phase disposable columns and then determined by reversed-phase ion-pairing high-performance liquid chromatography (HPLC) with selective colometric electrochemical detection, according to our procedures (15,16). The minimum urine levels detectable were 0.5, 1.0 and 3.0 nmol/l for norepinephrine, epinephrine and dopamine and 0.5, 0.5, 0.12 and 0.3 µmol/l for VMA, HVA, DOPAC and total MHPG. The percentage intra-assay (inter-assay) variability calculated at the concentrations found in the study were 2.4 (5.1), 2.6 (5.7) and 1.8 (3.6) for norepinephrine, epinephrine and dopamine, and 2.2 (4.7), 1.7 (3.5), 3.5 (7.5) and 4.2 (9.0) for VMA, HVA, DOPAC and total MHPG. Urine samples were expressed as nmol of catecholamines and as µmol of metabolites per gram of creatinine.

**Statistics**
Statistical analyses were performed by analysis of variance (ANOVA) and paired and unpaired Student’s t-test, as appropriate. Results are expressed in mean ± SEM.

**Table 3.**
Urinary levels of dopamine, norepinephrine, epinephrine, vanillylmandelic acid (VMA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), and total 3-methoxy-4-hydroxyphenylglycol (MHPG) in patients with idiopathic hyperprolactinemia before and during carbidopa (C-dopa) and disulfiram (TTD) administration. Data from control subjects are reported for comparison. Cr = Creatinine.

<table>
<thead>
<tr>
<th>Urinary catecholamines</th>
<th>Dopamine (nmol/g Cr)</th>
<th>Norepinephrine (nmol/g Cr)</th>
<th>Epinephrine (nmol/g Cr)</th>
<th>VMA (µmol/g Cr)</th>
<th>HVA (µmol/g Cr)</th>
<th>DOPAC (µmol/g Cr)</th>
<th>MHPG (µmol/g Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with idiopathic hyperprolactinemia (N=12)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2339.5</td>
<td>122.0</td>
<td>25.6</td>
<td>16.4</td>
<td>16.6***</td>
<td>3.84***</td>
<td>10.0</td>
</tr>
<tr>
<td>with C-dopa</td>
<td>235.1</td>
<td>15.2</td>
<td>4.2</td>
<td>1.5</td>
<td>1.6</td>
<td>0.39</td>
<td>1.0</td>
</tr>
<tr>
<td>Baseline</td>
<td>1199.9**</td>
<td>142.8</td>
<td>24.4</td>
<td>18.5</td>
<td>15.7***</td>
<td>3.92**</td>
<td>10.4</td>
</tr>
<tr>
<td>with TTD</td>
<td>2404.2</td>
<td>131.0</td>
<td>25.8</td>
<td>17.4</td>
<td>15.5***</td>
<td>3.90***</td>
<td>9.5</td>
</tr>
<tr>
<td>*</td>
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<td></td>
<td></td>
<td>**</td>
<td></td>
</tr>
<tr>
<td><strong>Control subjects (N=12)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EFP</td>
<td>1836.4</td>
<td>129.5</td>
<td>22.9</td>
<td>20.2</td>
<td>26.3</td>
<td>5.59</td>
<td>9.8</td>
</tr>
<tr>
<td>POP</td>
<td>2129.5</td>
<td>158.8</td>
<td>22.4</td>
<td>17.1</td>
<td>17.0</td>
<td>3.03</td>
<td>10.8</td>
</tr>
<tr>
<td>293.8</td>
<td>17.1</td>
<td>3.3</td>
<td>1.5</td>
<td>1.6</td>
<td>0.30</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean ± SEM, EFP, early follicular phase; POP, pre-ovulatory phase.
* p < 0.05, **p < 0.005, ***p < 0.001 compared with respective baseline value in patients with idiopathic hyperprolactinemia and EFP value in control subjects.

*p < 0.05, "p < 0.005, ""p < 0.001 compared with EFP value in control subjects.

*p < 0.05, "p < 0.005, ""p < 0.001 compared with POP value in control subjects.
Results
Baseline plasma PRL levels were markedly higher in the patients with idiopathic hyperprolactinemia than in control subjects in both phases of the cycle; they were not compared statistically because selected a priori. Plasma GH, TSH, testosterone and progesterone levels were similar, except that progesterone levels were higher in controls during the pre-ovulatory phase. LH, FSH and E2 levels in the patients were slightly and markedly lower than in controls in the early follicular phase and in the pre-ovulatory phase, respectively (Table 2). Baseline urinary dopamine, norepinephrine, epinephrine, VMA and MHPG levels did not differ significantly in patients and controls both in early follicular phase and pre-ovulatory phase. Urinary HVA and DOPAC levels were significantly lower in patients than in controls in the early follicular phase, whereas they were similar to those of controls in pre-ovulatory phase (Table 3).

During C-dopa administration, baseline plasma pituitary hormones and sex steroids and urinary catecholamine and metabolite levels were all unchanged in the patients, except for a marked decrease (about 50%) in unconjugated dopamine levels (Table 3).

During disulfiram administration, baseline plasma pituitary hormones and sex steroids were unchanged in the patients. Urinary dopamine, HVA and DOPAC levels increased and urinary norepinephrine, VMA and MHPG levels decreased significantly. Urinary epinephrine levels remained unchanged (Table 3).

In particular, by single distribution, baseline HVA levels were lower in the patients except in one, and baseline DOPAC levels showed only a small overlap compared with controls in the early follicular phase. On the contrary, a similar distribution for both metabolites was obtained in the patients and controls in the pre-ovulatory phase. During disulfiram administration both HVA and DOPAC levels increased in all patients and showed a distribution pattern similar and higher than that of the controls in the early follicular phase and in the pre-ovulatory phase, respectively (Fig. 1).

Discussion
Plasma pituitary hormone and sex steroid concentrations in the patients with idiopathic hyperprolactinemia are in accordance with those typical of the hyperprolactinemic states previously reported (17).

We chose urinary rather than plasma indices to

![Fig. 1](Distribution of urinary homovanillic acid (HVA) (left) and 3,4-dihydroxyphenylacetic acid (DOPAC) (right) levels in 12 patients with idiopathic hyperprolactinemia (IH) in basal conditions (B) and during disulfiram (TTD) administration compared with that of 12 control subjects (C) during the early follicular (EFP) and pre-ovulatory phases (POP) of the menstrual cycle. \( \overline{\Delta} \): mean \( \pm \) SEM. Cr=Creatinine.)
evaluate catecholamine activity, because, although they have insufficient temporal resolution to characterize acute changes accurately, they provide estimates of the overall catecholaminergic activity integrated over time (9) and avoid interferences owing to circadian variations in the levels of catecholamines, and their metabolites (8,18).

The similar values of urinary unconjugated catecholamines and VMA and MHPG in the patients and controls in the early follicular phase support a normal, global peripheral catecholamine and central norepinephrine activity. On the other hand, the lower values of the urinary metabolites HVA and DOPAC show a decreased central dopamine activity in patients with idiopathic hyperprolactinemia, confirming previous reports of decreased concentrations of dopamine at the median eminence level in rats exposed to elevated levels of PRL for long periods of time (19). Moreover, the decreased values of urinary HVA and DOPAC in idiopathic hyperprolactinemia are in good accordance with the similarly decreased values of the metabolites in controls in the pre-ovulatory phase (15). Thus, in the presence of unaltered values of all other catecholamines and metabolites, there is some resemblance between these two clinical entities (idiopathic hyperprolactinemia and normal women at mid-cycle) as regards a common deficiency in central dopamine activity. The E₂-induced PRL surge observed at mid-cycle in normal women (20) may probably explain the transient central dopamine deficiency in the pre-ovulatory phase.

C-dopa administration, by selective peripheral dopa-decarboxylase blockade (12), the enzyme that converts (L-)dopa to dopamine, induces a dramatic decrease (50%) only in unconjugated dopamine concentrations, confirming in idiopathic hyperprolactinemia the existence of important amounts of dopamine arisen from various peripheral sources (16). The lack of variation in HVA and DOPAC concentrations under C-dopa further supports their mainly CNS-derived dopamine source (7,8).

Disulfiram administration, by dopamine β-hydroxylase blockade (13,14), the enzyme that converts dopamine to norepinephrine, induces a significant increase in global dopamine excretion and a significant decrease in global norepinephrine excretion, supporting a dual effect of this inhibitor at both peripheral and central level, with no interferences in epinephrine excretion. The data fully agree with those of previous reports both in rats, where acute disulfiram administration induces a reduction in brain norepinephrine and a rise in brain dopamine (13), and in man, where disulfiram administration decreased urinary norepinephrine, VMA and MHPG excretion and increased urinary HVA excretion (14,21). Consequently disulfiram, increasing the HVA and DOPAC concentrations in the patients with idiopathic hyperprolactinemia to those of the controls in the early follicular phase, normalize the baseline deficient endogenous central dopamine activity (Fig. 1). That could probably explain the slight (10%) and not significant decrease in serum PRL (Table 2), already observed under disulfiram in normal men (22) and in women with polycystic ovaries (21).

Despite the obvious advantages of the non-invasive techniques adopted for measuring catecholamines and their metabolites, our data are unable to establish the precise biochemical defect responsible for the central dopamine deficiency and must be considered with caution for several reasons, as previously observed (15,16). On the other hand, direct invasive studies, collecting hypothalamic-hypophysial portal blood for catecholamine assays are practicable only during transsphenoidal microsurgery for tumours, according to our procedure (23,24), but obviously not in idiopathic hyperprolactinemia.

On the whole, this study confirms the presence of a defect in CNS-dopamine neurotransmission, as previously suggested (1,3,4) and offers the first direct evidence of a pathological reduction of CNS-dopamine metabolism in human idiopathic hyperprolactinemia. It is a matter of speculation whether our findings are the result rather than the cause of the mechanisms activating hyperprolactinemia, however, the "quantitatively" low central dopamine activity may have a pivotal role in the pathogenesis of idiopathic hyperprolactinemia.

Acknowledgments

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