TYROSINE ADMINISTRATION DECREASES SERUM PROLACTIN LEVELS IN CHRONICALLY RESERPINIZED RATS

Alan F. Sved, John D. Fernstrom, and Richard J. Wurtman*

Laboratory of Brain and Metabolism
and
*Laboratory of Neuroendocrine Regulation
Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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Summary

The injection of tyrosine, 200 mg/kg, decreased serum prolactin levels and elevated hypothalamic (and striatal) concentrations of two dopamine metabolites, dihydroxyphenylacetic acid and homovanillic acid, in chronically reserpinized rats. Tyrosine administration had none of these effects in otherwise untreated rats, and did not block the increase in serum prolactin that occurred 4 hours after a single injection of reserpine. As anticipated, the injection of dopa decreased serum prolactin in all rats. Valine, another large neutral amino acid, did not modify serum prolactin in chronically reserpinized animals. Since prolactin secretion is normally inhibited by dopamine released from the hypothalamus, reserpine treatment probably elevates serum prolactin by depleting the hypothalamus of dopamine. Our data suggest that tyrosine injection suppresses serum prolactin levels in chronically reserpinized rats by enhancing the synthesis and release of hypothalamic dopamine. Thus, administration of tyrosine, dopamine's dietary precursor, can alter physiologic functions that depend on dopamine.

The synthesis and release of dopamine and norepinephrine within the rat's brain depend in part on the availability of the precursor amino acid, tyrosine. Systemic administration of tyrosine (a) increases the rate at which dopa accumulates in the brains of rats given a centrally acting decarboxylase inhibitor (1,2); (b) elevates brain methoxyhydroxyphenylethlamineol-sulfate (MOPEGSO₄) levels in rats pretreated with probenecid or exposed to low (4°C) ambient temperatures (3) and in spontaneously hypertensive...
rats (4); and (c) raises striatal homovanillic acid (HVA) levels in rats pretreated with haloperidol (5), a drug that blocks dopamine receptors and thereby accelerates the firing rate of nigrostriatal neurons.

We recently observed that administration of tyrosine to spontaneously hypertensive rats, in doses that elevate brain tyrosine and MOPBG-SO₄ levels, causes a large reduction in blood pressure (4). This finding suggests that tyrosine-induced stimulation of brain norepinephrine (or epinephrine) formation can influence brain outputs that depend on central noradrenergic neuron activity. Although brain tyrosine levels influence a drug-induced brain output involving dopamine neurons (the fall in body temperature caused by amphetamine administration to rats [6]), few data are available on tyrosine administration's effects on physiologic functions mediated by dopamine neurons.

We now report that tyrosine injection can affect a process thought to be controlled in part by dopamine release from tuberoinfundibular neurons. Tyrosine administration to rats made hyperprolactinemic by repeated injections of reserpine rapidly suppresses serum prolactin, and also raises levels of the dopamine metabolites HVA and dihydroxyphenylacetic acid (DOPAC) in the hypothalamus and striatum.

Materials and Methods

Male Sprague-Dawley rats weighing 250 g (Charles River Breeding Laboratories, Wilmington, MA) were housed in groups of 4-5 in our animal facilities. Food (Charles River Rat, Mouse, and Hamster Maintenance Formula) and water were provided ad libitum, and the animals were exposed to 12 hours of light each day (Vita-Lite, 300 µW/cm², Duro-Test Corp., North Bergen, NJ). The ambient temperature was maintained at 22°C.

Reserpine (Sigma Chemical Co., St. Louis, MO) was administered intraperitoneally in 1% ascorbic acid; L-tyrosine, L-valine (ICN Nutritional Biochemicals, Cleveland, OH), and L-dopa (gift of Hoffman-LaRoche, Nutley, NJ) were injected intraperitoneally, suspended in deionized water. Chronically reserpinized rats received 2.5 mg/kg reserpine twice daily for 4 days. On the fifth day, they received tyrosine (200 mg/kg = 1.1 mmol/kg), dopa (1.1 mmol/kg), valine (1.1 mmol/kg), or the vehicle (deionized water, 2 ml/kg) 2 hours after the morning dose of reserpine, and were killed 1 hour later. Acutely reserpinized rats received the amino acid (1.1 mmol/kg) 4 hours after a single reserpine injection (10 mg/kg), and were killed 1 hour later.

Animals were killed by decapitation, and blood was collected from the cervical wound and centrifuged. The sera were stored frozen at -20°C until they could be assayed for prolactin. Serum prolactin concentrations were estimated by a double-antibody radioimmunoassay using materials provided by the National Institute of Arthritis, Metabolism, and Digestive Diseases, Rat Pituitary Program. Rat prolactin RP-1 (National Institutes of Health) was the reference preparation.

DOPAC and HVA were measured using a high-performance liquid chromatograph (HPLC) coupled to an electrochemical detector.
(Bioanalytical Systems, West Lafayette, IN) (Hefti, submitted for publication). Samples were homogenized in 1.2 ml of 0.1 M perchloric acid and centrifuged. HVA and DOPAC were extracted from 0.5 ml of the supernatant fluid into ether. The ether layer was then transferred to clean tubes and lyophilized. The samples were reconstituted in 100 μl of 0.05 M acetate buffer, pH 5.0, and portions were injected onto the column (Bondapak C18, Waters Assoc., Milford, MA). The column buffer (0.05 M acetate, pH 5.0) was run at 1.5 ml/min at room temperature; retention times were 6.2 min and 18.8 min for DOPAC and HVA, respectively. This procedure can detect as little as 0.2 ng of each metabolite. Peak heights were used to generate standard curves, which were linear from 0.2 ng to at least 100 ng. Dopamine was also assayed by HPLC-electrochemical detection, using a phosphate buffer system as described previously (7).

Data were analyzed by analysis of variance and the Newman-Keuls test (8).

Results

As anticipated (9), both acute and chronic treatment with reserpine elicited large increases in serum prolactin levels, and dopa administration caused a large drop in serum prolactin (Table 1). Tyrosine administration did not affect serum prolactin levels in otherwise untreated rats or in animals given a single reserpine injection; however, in four separate sets of experiments, the amino acid caused a large reduction in serum prolactin among animals chronically pretreated with reserpine (Table 1). Injection of valine, a large neutral amino acid that is not a dopamine precursor, into chronically reserpinized rats did not reduce serum prolactin (Table 1). Thus, tyrosine's effect on serum prolactin levels is not a non-specific consequence of amino acid administration.

Tyrosine also increased the levels of dopamine metabolites in the hypothalami of chronically reserpinized rats; both DOPAC and HVA were increased by about 40% (P < 0.05) (Table 2). (Dopamine levels were undetectable in hypothalami of chronically reserpinized rats, whether or not the animals also received tyrosine.) DOPAC and HVA levels in striata of chronically reserpinized rats also increased after tyrosine administration, but those in animals not receiving reserpine did not (Table 2).

Discussion

These data demonstrate that, in rats treated chronically with reserpine, tyrosine administration can modify prolactin secretion, a brain output thought to be controlled in part by dopamine release from tubero-infundibular neurons. Presumably, the mechanism through which tyrosine suppresses serum prolactin involves acceleration of dopamine synthesis in, and release from, these tubero-infundibular neurons. Once released into the pituitary portal circulation, dopamine has a direct inhibitory effect on prolactin secretion from the pituitary (10). A similar mechanism is commonly invoked to explain dopa's suppression of prolactin secretion (11), that is, a direct action on the pituitary by dopamine formed from the dopa. Of course, other mechanisms might also mediate tyrosine's effect on prolactin secretion. For example, enhanced peripheral synthesis and release of dopamine, norepinephrine, and epinephrine in response
to tyrosine might directly inhibit prolactin secretion: we have observed (12) significant increases in all three catecholamines in urine samples from rats given tyrosine. Moreover, Shaar and Clemens (13) have reported that norepinephrine and epinephrine (in addition to dopamine) can directly inhibit prolactin release from the pituitary (at least in vitro).

Exogenous dopa is metabolized to dopamine within most cells in the brain and elsewhere, while tyrosine is converted to dopamine only within cells that normally produce catecholamines. Moreover, exogenous tyrosine's ability to enhance catecholamine synthesis within such cells seems to vary in relation to their physiological activity (14). This difference may explain why dopa invariably suppressed serum prolactin levels in all groups of animals, while tyrosine did so only in chronically reserpinized rats; the exogenous dopa always caused more dopamine to impinge on the pituitary, while the exogenous tyrosine did so only after a treatment (chronic reserpine) that heightened the susceptibility of dopamine-producing.

### TABLE 1

Effect of Tyrosine Injection on Serum Prolactin Levels in Reserpinized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serum Prolactin (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Pretreatment</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>52 ± 13</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>49 ± 9</td>
</tr>
<tr>
<td>Dopa</td>
<td>33 ± 8*</td>
</tr>
<tr>
<td>Acute Reserpine</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>145 ± 37</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>125 ± 35</td>
</tr>
<tr>
<td>Dopa</td>
<td>40 ± 10*</td>
</tr>
<tr>
<td>Chronic Reserpine</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>156 ± 14</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>80 ± 8*</td>
</tr>
<tr>
<td>Dopa</td>
<td>43 ± 3*</td>
</tr>
<tr>
<td>Valine</td>
<td>121 ± 39</td>
</tr>
</tbody>
</table>

Groups of 4-5 rats received vehicle, tyrosine (200 mg/kg), dopa (217 mg/kg), or valine (129 mg/kg), and were killed 1 hour later. Animals had previously received one of the following treatments: none, acute reserpine (10 mg/kg, 4 hours before amino acid injection), or chronic reserpine (2.5 mg/kg twice daily for 4 days; on the morning of the fifth day, the last injection was given 2 hours before amino acid administration).

*P < 0.05, compared to vehicle values within the same pretreatment group.
cells to precursor control, by increasing their physiological activity.

TABLE 2

Effect of Tyrosine Injection on Striatal and Hypothalamic Levels of Dopamine Metabolites in Reserpinized Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Striatum DOPAC (ng/g, wet weight)</th>
<th>Striatum HVA (ng/g, wet weight)</th>
<th>Hypothalamus DOPAC (ng/g, wet weight)</th>
<th>Hypothalamus HVA (ng/g, wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Reserpine</td>
<td>437 ± 38</td>
<td>472 ± 19</td>
<td>56 ± 18</td>
<td>187 ± 20</td>
</tr>
<tr>
<td>Vehicle</td>
<td>438 ± 26</td>
<td>412 ± 68</td>
<td>85 ± 21</td>
<td>243 ± 35</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>601 ± 70</td>
<td>736 ± 22</td>
<td>78 ± 8</td>
<td>386 ± 29</td>
</tr>
<tr>
<td>Chronic Reserpine</td>
<td>850 ± 41*</td>
<td>1025 ± 68*</td>
<td>336 ± 126*</td>
<td>595 ± 109*</td>
</tr>
</tbody>
</table>

Groups of 5 rats received vehicle or tyrosine (200 mg/kg), and were killed 1 hour later. Animals had been pretreated with either nothing (No Reserpine) or reserpine (Chronic Reserpine), 2.5 mg/kg twice daily for 4 days; on the morning of the fifth day, the last injection was given 2 hours before amino acid administration. All injections were intraperitoneal. Data are presented as means ± standard errors. *P < 0.05, compared to vehicle values within the same pre-treatment group.

The finding that tyrosine elevated hypothalamic and striatal DOPAC and HVA levels in chronically reserpinized rats but not in control animals concurs with the hypothesis that the amino acid stimulates dopamine synthesis and release in the former group, but not in the latter. Pretreatment of rats with haloperidol, a drug that blocks dopamine receptors, also causes dopamine synthesis to become precursor-dependent, and accelerates dopamine release after tyrosine administration (5). This finding is consistent with the hypothesis that treatments that accelerate the firing of dopaminergic neurons enhance exogenous tyrosine’s ability to stimulate dopamine synthesis, possibly by changing the kinetic properties of tyrosine hydroxylase. Chronic reserpine treatment, by depriving dopamine receptors of transmitter, might similarly increase the firing rates of dopamine neurons as well as their precursor dependence; this view is supported by the observation that tyrosine administration increased striatal and hypothalamic DOPAC and HVA levels in chronically reserpinized animals but not in untreated animals. (Of course, data on hypothalamic DOPAC and HVA levels may only partly reflect dopamine release from median eminence neurons. Unfortunately, treatments that alter dopamine turnover in the median eminence (15,16) apparently fail to produce parallel changes in median eminence DOPAC levels, even though dopamine turnover elsewhere in the brain is associated with changes in DOPAC. The most meaningful index of dopamine release in the median eminence thus
may be changes in serum prolactin levels; these fall in chronically reserpinized rats receiving tyrosine, but not in control animals or acutely reserpinized animals injected with tyrosine. Presumably, these changes indicate that dopamine release in the median eminence is not accelerated in these latter animals.)

The failure of tyrosine administration to reduce elevated serum prolactin levels caused by acute reserpine treatment is consistent with recent observations that tubero-infundibular dopamine neurons differ from nigrostriatal dopamine neurons in requiring relatively long periods to manifest responses to several drugs. For example, haloperidol treatment increases striatal dopamine turnover within 1 hour, but requires 16 hours to affect dopamine turnover in the median eminence (17). Thus, if tyrosine treatment increases dopamine release only from rapidly firing neurons, tyrosine would be ineffective in lowering serum prolactin levels when given 4 hours after a single dose of reserpine, but might be effective when given to animals chronically exposed to the drug.

Moore et al. (17-20) suggested that the increase in serum prolactin after haloperidol treatment mediates the increase in median eminence dopamine turnover; the high serum prolactin levels feed back on the tubero-infundibular dopamine neurons to increase their activity. Indeed, hyperprolactinemia, whatever its cause, apparently increases dopamine release by tubero-infundibular dopamine neurons (21-23). Conceivably, hyperprolactinemia is a critical mediator of reserpine's ability to render tubero-infundibular dopaminergic neurons susceptible to exogenous tyrosine.

Clinically, hyperprolactinemia of various etiologies (including hyperprolactinemia following chronic use of reserpine) may be associated with an increased incidence of certain types of cancer (24), and with amenorrhea (25), galactorrhea (26), or impotence (27). Several dopaminergic agonists are available for treating hyperprolactinemia and its symptoms (28), however, these agents all produce side effects because they stimulate other dopamine receptors in addition to those on pituitary prolactin-secreting cells. If tyrosine is useful in treating clinical hyperprolactinemia, it might, in certain circumstances, have more specificity than the dopamine agonists: in situations in which the hyperprolactinemia has activated only those dopamine neurons in the median eminence (21-23), tyrosine might also enhance dopamine release only from those neurons.

References