Dopamine in the nucleus accumbens: preferential increase of DA turnover by rat prolactin

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A number of investigations have shown that dopamine (DA) in the median eminence is involved in the inhibitory control of prolactin secretion (see ref. 17). The studies, particularly those by MacLeod and co-workers16, also indicate that DA may be released into the portal vessels to act as a prolactin inhibitory factor by binding to receptors of prolactin-containing pituitary cells. Using semiquantitative estimations and also quantitative microfluorimetric measurements of DA fluorescence in the median eminence, it was established that single or repeated injections of high doses (1–10 mg/rat, 24 h intervals) of ovine prolactin markedly increased DA turnover in the medial and lateral palisade zones (MPZ and LPZ) of the median eminence, particularly in hypophysectomized rats12. These results suggest that DA terminals in the median eminence mediate the inhibitory feedback action of prolactin on its own secretion. At the same time some data7,10 have indicated that DA nerve terminals in the median eminence also inhibited the secretion of luliberin.

In the present study the aim has been to try to differentiate which of the median eminence DA nerve terminals are involved in the control of prolactin secretion. The working hypothesis is that LPZ DA nerve terminals are mainly involved in the control of luliberin secretion and not of prolactin secretion, since the luliberin-containing nerve terminals are highly concentrated in this region13. Therefore, it has been of special interest to study the catecholamine (CA) fluorescence in MPZ of rats treated with rat prolactin. The effect of rat prolactin on telencephalic DA nerve terminals has also been studied since lesions in the posterior and dorsal part of the nuc. accumbens have resulted in reduction of postpartum behaviour and impairment of lactational performance20. DA nerve terminals in the nuc. accumbens are so far known to be involved in locomotor performance19. Radioimmunoassay determinations of prolactin levels have also been performed in order to correlate regional CA turnover in brain directly with serum prolactin levels.

Male Sprague–Dawley rats (150 g body weight) were used. Hypophysectomy was performed one month before the experiment using the parapharyngeal approach.
The rats were kept on a standard light–dark schedule (light: 06.00–20.00 h) and had free access to food and to a 5 % (w/v) glucose–0.9 % (w/v) saline solution. CA turnover changes were measured by studying the changes in the disappearance of regional CA stores after tyrosine hydroxylase inhibition using α-methyl-tyrosine methylester (H44/68)\textsuperscript{1}. For treatment schedule, see text to Table I.

After the routine Falck–Hillarp procedure\textsuperscript{11} the DA fluorescence in the following regions was measured by means of quantitative microfluorimetry\textsuperscript{5,14,15}: nuc. caudatus\textsuperscript{6}, tuberculum olfactorium (diffuse fluorescence)\textsuperscript{6,18}, nuc. accumbens (diffuse fluorescence, posterior half)\textsuperscript{2,6,18}, and LPZ\textsuperscript{6,14}. The CA fluorescence in the MPZ (mixture of DA and noradrenaline (NA) nerve terminals in the approximate ratio of 1.5:1)\textsuperscript{13,14} and in the subependymal layer (SEL) (mainly NA terminals)\textsuperscript{13,14} was also measured. For further details on the quantitative microfluorimetric procedure, see refs. 5, 14 and 15. For statistical procedures, see legends to Tables I and II.

Rat prolactin (7 IU/mg* as compared to at least 25 lU/mg for pure rat prolactin) and the kits for radioimmunoassay of rat prolactin, rat lutropin and rat follitropin were kindly supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases (NIAMDD), Rat Pituitary Hormone Distribution Program. The hormones were iodinated according to Bolton and Hunter\textsuperscript{9}. The assays were principally carried out in accordance with NIAMDD instructions.

### TABLE I

**Effect of hypophysectomy with and without rat prolactin treatment on the CA turnover in various brain regions of male rats and correlation with serum prolactin levels**

Hypophysectomy (Hypox) was performed 4 weeks earlier. Rat prolactin (PRL, kindly supplied by NIAMDD-Rat Pituitary Hormone Distribution Program) was given i.v. in a dose of 100 μg/kg 2 h before α-methyl-tyrosine methylester (H44/68, 250 mg/kg, i.p., 2 h before decapitation). The CA fluorescence values in the various regions are given as median ± semi-quartile deviation in per cent of respective untreated group mean value. Number of rats in parentheses. Statistical analysis was made according to the Kruskal–Wallis non-parametric analysis of variance followed by Tukey’s Quick Test. $r_s$ = Spearman’s rank correlation coefficient between serum prolactin levels and regional brain CA concentrations 2 h after H44/68 (for interpretation, see text). Abbreviations: SEL, subependymal layer of the median eminence; MPZ, medial palisade zone of the median eminence; LPZ, lateral palisade zone of the median eminence; TO, tuberculum olfactorium; ACC, nuc. accumbens; CAUD, nuc. caudatus.

<table>
<thead>
<tr>
<th></th>
<th>SEL</th>
<th>MPZ</th>
<th>LPZ</th>
<th>TO</th>
<th>ACC</th>
<th>CAUD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control + H44/68</td>
<td>44 ± 9 (3)</td>
<td>41 ± 8 (3)</td>
<td>46 ± 11 (3)</td>
<td>64 ± 9 (4)</td>
<td>52 ± 11 (4)</td>
<td>69 ± 12 (4)</td>
</tr>
<tr>
<td>Hypox + H44/68</td>
<td>53 ± 16 (4)*</td>
<td>54 ± 10 (4)</td>
<td>88 ± 16 (4)*</td>
<td>66 ± 9 (4)</td>
<td>48 ± 12 (4)</td>
<td>57 ± 5 (4)</td>
</tr>
<tr>
<td>$r_s$</td>
<td>−0.05</td>
<td>+0.65*</td>
<td>−0.05</td>
<td>+0.65</td>
<td>+0.35</td>
<td>+0.55</td>
</tr>
<tr>
<td>Hypox + H44/68  + PRL</td>
<td>65 ± 22 (5)*</td>
<td>57 ± 14 (5)</td>
<td>83 ± 2 (5)*</td>
<td>70 ± 12 (5)</td>
<td>33 ± 14 (5)**</td>
<td>65 ± 8 (5)</td>
</tr>
<tr>
<td>$r_s$</td>
<td>−0.60</td>
<td>−0.70*</td>
<td>+0.20</td>
<td>−0.38</td>
<td>−0.70*</td>
<td>−0.53</td>
</tr>
</tbody>
</table>

* $= P < 0.05$ versus control. ** $= P < 0.05$ versus non-prolactin treated groups.

$* = P < 0.10$ according to statistical tables for $r_s$.

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* This preparation was not altogether homogeneous on SDS electrophoresis.
TABLE II
Prolactin serum levels after treatment of hypophysectomized male rats with rat prolactin

Rat prolactin was given i.v. in a dose of 100 μg/kg 2 h before H44/68 (250 mg/kg, i.p., 2 h before decapitation). Prolactin was determined by radioimmunoassay (see text). Means ± S.E.M. (μg/ml). Number of rats in parentheses. Statistical testing was made according to Kruskal–Wallis analysis of variance. Follitropin and lutropin levels were not detectable in any of the groups. No significant differences were found.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (μg/kg)</th>
<th>Serum prolactin levels (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>16 ± 4 (4)</td>
</tr>
<tr>
<td>Rat prolactin</td>
<td>100</td>
<td>23 ± 9 (4)</td>
</tr>
<tr>
<td>H44/68</td>
<td></td>
<td>17 ± 1 (4)</td>
</tr>
<tr>
<td>Rat prolactin + H44/68</td>
<td>100</td>
<td>31 ± 8 (5)</td>
</tr>
</tbody>
</table>

The CA fluorescence in the hypophysectomized rats was not significantly affected by the rat prolactin treatment 2 or 4 h prior to decapitation in any of the areas studied (data not shown). The H44/68-induced CA fluorescence disappearance, however, was significantly increased in the nuc. accumbens but not in MPZ or in LPZ, nor in any other region studied (Table I). As shown in Table I, the CA terminals in LPZ, but not in MPZ, of hypophysectomized rats showed a significantly reduced fluorescence disappearance after H44/68 treatment compared to H44/68-treated control rats. A similar result was obtained in studies on the NA fluorescence in SEL, in agreement with previous biochemical studies on NA turnover in whole brain. The reduction of H44/68-induced NA fluorescence disappearance was not counteracted but rather further reduced by the prolactin treatment (Table I). This latter finding does not support an involvement of NA in the inhibitory feedback action of prolactin on its own secretion.

The serum prolactin levels in the animals are given in Table II. No significant differences were found. Treatment with H44/68 did not increase prolactin levels in the hypophysectomized animals, indicating reduced hypothalamic control of prolactin levels in hypophysectomized rats. Thus, in a separate experiment, treatment of normal male rats with H44/68 in an identical manner to the experiments described in this investigation led to a marked increase in serum prolactin levels from 8 ± 1 μg/l (mean ± S.E.M.; n = 4) to 101 ± 8 μg/l (n = 4) (cf. ref. 4). The reason why the basal serum levels of prolactin were not reduced by hypophysectomy may have been that a very small part of the anterior pituitary remained after operation and that this remaining piece of the gland, free from central inhibitory control, secreted enough prolactin to maintain normal serum prolactin levels, in spite of the fact that follitropin and lutropin levels were not detectable. The possibility that prolactin or prolactin-like material might be synthesized elsewhere outside the pituitary gland cannot be excluded.

Trends for significant intraindividual correlations between serum prolactin levels and CA fluorescence in hypophysectomized rats after treatment with prolactin and
H44/68 were obtained in MPZ and nuc. accumbens but not in any other regions. In both MPZ and nuc. accumbens a negative correlation was obtained, i.e. the higher the prolactin level, the lower the CA fluorescence after H44/68 treatment. On the other hand, in the hypophysectomized rats treated with H44/68 alone, a trend for a significant positive correlation was obtained in the MPZ, i.e. the higher the prolactin level, the higher the CA fluorescence after H44/68 treatment, whereas no correlation was obtained in nuc. accumbens.

The fluorescence measured in nuc. accumbens was the diffuse DA fluorescence\(^\text{18}\) dorsomedial to the anterior commissure in the posterior half of nuc. accumbens. The observation that administration of rat prolactin increases DA turnover in this region of the brain (Table I) may indicate that DA in posterior nuc. accumbens participates in the control of prolactin secretion. This control seems to be inhibitory, since the correlation coefficient shows that the higher the prolactin level, the higher is the DA turnover in nuc. accumbens after treatment of hypophysectomized rats with rat prolactin. It may, therefore, be speculated that DA in nuc. accumbens is involved in mediating the inhibitory feedback action of prolactin on its own secretion. This mechanism mainly seems to operate when excess prolactin is present since the correlation did not exist in the experimental group not given prolactin (Table I). These results are in agreement with the findings of Smith and Holland\(^\text{30}\). It may be that DA in posterior nuc. accumbens directly or indirectly controls pathways containing prolactin releasing factor(s). Finally, the possibility should be considered that prolactin also increases DA turnover in the DA terminals of the nuc. accumbens that are involved in the control of locomotion\(^\text{19}\). Thus, prolactin may influence behaviour.

In view of the established existence of an inhibitory dopaminergic mechanism in the median eminence controlling prolactin secretion, it may at first seem surprising that the dose of rat prolactin used in the present investigation (100 μg/kg, i.v.) neither affected the DA turnover in the LPZ, nor the CA turnover in the MPZ which contains a mixture of DA and NA nerve terminals\(^\text{13,14}\). However, the fact that the correlation coefficient between serum prolactin levels and CA fluorescence in MPZ of hypophysectomized and H44/68-treated rats changed from +0.65 to −0.70 following treatment with rat prolactin does in fact suggest a possible inhibitory involvement of MPZ DA in control of prolactin secretion (cf. above). The failure to see any significant effects of prolactin treatment on CA turnover in MPZ may be explained on the basis that only the time 2 h after treatment with H44/68 was studied, since the DA turnover in this region can be expected to be very high.

The demonstration of a reduction of DA turnover in the LPZ but not MPZ after hypophysectomy, which was unaffected by treatment with rat prolactin, is in agreement with the existence of two types of DA nerve terminals in the median eminence, one inhibiting luteinizing hormone secretion (LPZ), and one inhibiting prolactin secretion (MPZ). The reason why CA turnover in the MPZ was not reduced following hypophysectomy may have been the near normal levels of serum prolactin in hypophysectomized rats (cf. discussion above). Recently, nerve terminals containing prolactin immunoreactivity have been described in the hypothalamus and the preoptic area, especially in the periventricular
region. It remains to be shown whether these terminal networks may also somehow be involved in the inhibitory feedback action of prolactin on its own secretion. Thus, it may well be that the hypothalamic prolactin-containing nerve terminals are associated with the dendrites of the DA cell bodies in the medio-basal hypothalamus.

In conclusion, the present results indicate that rat prolactin preferentially increases DA turnover in the posterior nuc. accumbens of hypophysectomized male rats. It is speculated that DA can be involved in the inhibitory control of prolactin secretion, and that prolactin can cause behavioural effects, for example on postpartum behaviour, by affecting DA systems in the nuc. accumbens.

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