CHAPTER 3

REGULATION OF ORNITHINE DECARBOXYLASE BY PROLACTIN IN THE KIDNEY AND LIVER OF RAT
Prolactin has been most widely recognised for its lactogenic properties and its role in the regulation of mammary function. However, in the course of studies involving this hormone, it has been shown that PRL has many diverse effects in various tissues. In addition to its mammotrophic properties, it is involved in functions such as growth promotion, osmoregulation, immunomodulation, liver mitogenesis and blood pressure regulation. PRL is an important trophic hormone in rat liver. Administration of PRL induces hepatic ODC activity, DNA synthesis, hypomethylation of DNA and causes hepatomegaly. Mammalian kidneys contain receptors for PRL. Male rat kidneys have been shown to have a higher number of PRL receptors than those of female rats. PRL modulates renal polyamine and cyclic AMP formation. PRL also triggers the expression of genes which encode growth-related proteins such as insulin-like growth factor and Nb29, a 70 kDa heat shock protein-like molecule.

Prolactin induction can be exerted at multiple levels following PRL-binding to cell surface receptors, but neither the intracellular signalling pathway(s) nor the mechanism is known. It has been demonstrated that induction of milk protein gene expression by prolactin is effected via a serine/threonine kinase and possibly a tyrosine kinase. Various studies have implicated protein kinase C activation as a possible mechanism via which some of the actions of PRL are mediated. Various other mechanisms like stathmin phosphorylation, stimulation of a G protein which is not Gs, and even a direct translocation into the nucleus of a PRL-receptor complex to causing direct stimulation of
transcription, have been suggested.

In this study, an attempt has been made to investigate the mechanism by which PRL influences ornithine decarboxylase activity and gene expression in the liver and kidney of rat.

MATERIALS AND METHODS:

The general methods used have been described in Chapter 2. All the experiments in this chapter involved the use of adult male rats of 300-350 g. body weight. The rats were castrated via the scrotal route under mild ether anesthesia, and were used 4 to 5 days after castration.

Ovine prolactin was dissolved just prior to use in 0.9% sodium chloride/ 0.1 N NaOH, and the pH was adjusted to 7.5. The dose of PRL used throughout was 5.5 mg/kg body weight, and injections were administered intraperitoneally in 200 μl of the vehicle. Controls were given an equal volume of vehicle alone.

Actinomycin D was given intraperitoneally at a dosage of 6 mg/kg body weight, dissolved in ethanol/DMSO (1:1). It was injected 1h before administration of the hormone. Cycloheximide was injected intraperitoneally at a dosage of 8 mg/kg body weight, 1h before the rats were killed. Quercetin dissolved in ethanol was injected intraperitoneally at a dosage of 10 μmoles or 100 μmoles/kg body weight; 15 minutes before injection of PRL. Quinacrine was injected intra peritoneally at a dosage of 200 μmoles/ kg body weight, 15 minutes before injection of PRL.
The processing of tissue, RNA isolation and hybridisation, and ODC enzyme assay have been described in Chapter II.

RESULTS:

The effect of a single injection of PRL on renal ODC activity is shown in Table 1. The ODC activity increased with time and peaked at 4-6 hours after injection, after which it fell slightly at 7h. The time course of induction of hepatic ODC activity by PRL is given in Table 2. This followed a pattern similar to that shown by renal ODC, except that by 7h the activity had declined to control levels. Also, the basal ODC activity in control groups and the extent of induction by PRL was greater in kidney than in the liver.

Fig. 1 shows the time course of induction of renal ODC mRNA levels in response to PRL, as studied by northern blot hybridisation. ODC mRNA levels peaked at 2h after injection, and were lowered by 6h. An interesting observation was that in orchidectomised rats, the induction of ODC mRNA was higher than in intact rats. At 6h post-injection, ODC mRNA levels were still elevated as compared to intact rats. In subsequent experiments castrated rats treated for 6h with PRL were used. Fig. 2 shows the northern blot of ODC mRNA levels in liver in response to PRL. Two peaks of high mRNA levels were seen, at 1h and 6h after injection. By 7h after injection, the mRNA levels had declined.

The effect of simultaneous injections of testosterone
propionate (TP) and PRL on renal ODC activity is shown in Table 3. At 6h post-injection, the ODC activity was elevated considerably beyond the activity seen in groups treated with PRL or TP alone. The effect of the same treatment on ODC mRNA levels is shown in Fig. 3. It was seen that at 6h after injection, ODC mRNA levels are considerably reduced as compared to those of the groups treated with TP or PRL alone.

Table 4 shows the effect of pre-treatment with actinomycin D, a transcription-blocking agent, on PRL induction of renal ODC activity. It was observed that the ODC activity was totally suppressed by actinomycin D.

The effect of pre-treatment with actinomycin D on PRL induction of renal ODC mRNA levels is shown in Fig. 4. ODC mRNA levels were suppressed by the presence of actinomycin D to about 40% that of groups treated with PRL alone.

The effect of the protein translation inhibitor cycloheximide on ODC activity in PRL-treated kidney is given in Table 5. Rats were given PRL 6h prior to sacrifice and cycloheximide 1h prior to sacrifice. Cycloheximide treatment was seen to completely abolish the induction of ODC activity by the hormone. The same treatment caused an increase in ODC mRNA levels in the group treated with both PRL and cycloheximide (Fig. 5).

The effect of similar cycloheximide treatment on ODC activity in liver is shown in Table 6, where it can be seen that
the activity is totally abolished as in the case of the kidney. Similarly, as shown in Fig. 6, an increase in ODC mRNA levels was seen in the group treated with both PRL and cycloheximide.

Quercetin, a protein kinase C inhibitor, when administered just prior to PRL caused almost total blockage of PRL-induction of renal ODC activity, as shown in Table 7. A lower dose of 10 μM quercetin slightly inhibited ODC activity, whereas the higher dose of 100 μM caused a much higher level of inhibition. However, as shown in Fig. 7, there was no inhibition of ODC mRNA expression by quercetin.

Table 8 shows the effect of quercetin on liver ODC activity. In this case too, pre-treatment with quercetin totally inhibited PRL-induced ODC activity.

Table 9 shows the effect of a phospholipase inhibitor, quinacrine, on the PRL-induced ODC activity. Pre-treatment with quinacrine, like that with quercetin, inhibited the ODC activity. This effect was more pronounced in the liver (Table 10) than in the kidney. In the kidney, quinacrine treatment caused a pronounced increase in PRL-treated ODC mRNA levels (Fig. 9). A similar effect, however, was not observed in the liver (Fig. 10).
DISCUSSION:

The rapid and transient nature of ODC induction by mitogens associates it with growth regulatory phenomena. In any given system in which ODC activity can be made to change, those changes may be dependent to a greater or lesser extent on alterations in mRNA levels. In some cases (Feinstein et al., 1985) it was shown that changes in ODC mRNA and enzymatic activity are similar in timing and in extent, whereas in others, the magnitude of induction of activity exceeded that of mRNA (Katz and Kahana, 1987).

Various hormones have been shown to modulate ODC activity in the liver and kidney as well as other tissues. These include parathyroid hormone, calcitonin, vasopressin, d-aldosterone and L-triiodothyronine which increase renal ODC activity (Aragona et al., 1976). Prolactin has been shown to increase ODC activity in various tissues including mammary gland, liver, kidney, brain and testis. Prolactin also regulates various cellular functions.

The present study shows that both ODC enzyme activity and mRNA levels are regulated by prolactin in the liver and kidney of male rats. Previous studies have all involved usage of large doses of the hormone (~ 22 mg/kg body weight). In 1986, Buckley et al showed that a dose of 5.5 mg/kg body weight was able to stimulate rat hepatic DNA synthesis. Subsequently it was shown that the same dose of PRL caused significant hypomethylation of DNA in both the liver and kidney of rat (Reddy and Reddy, 1990). This study shows that the same low dose of PRL significantly
increases ODC activity and mRNA levels in these organs. A single injection of PRL induced both ODC mRNA levels and enzyme activity; however, the extent of induction of activity was by far the greater, and the peak of enzyme activity lagged behind that of the mRNA levels. An interesting observation was that in castrated rats, the basal ODC activity in kidney was higher than that in intact rats.

The extent to which PRL induction of renal ODC activity took place was greater in intact than in castrated rats. However, ODC mRNA levels in PRL-treated castrated rats were higher than in PRL-treated intact rats. This could indicate a post-transcriptional level of regulation in castrated rats. It also suggests a possible antagonistic effect of testosterone on renal ODC induction by PRL. It has been observed by Aragona et al (1976) that in rat liver, there is an increased binding of PRL to its receptors after castration, and that this effect is prevented upon treatment with testosterone.

In this study, when castrated rats were injected with testosterone propionate, PRL, or a combination of both hormones (Table 3) it was seen that at 6 h post-injection, groups receiving both hormones had an elevated ODC activity as compared to that of groups treated with either one of the hormones. Examination of the RNA levels showed that these were lowered at 6h in groups given both hormones as compared to those given either hormone alone (Fig. 3). The reason for this is not clear but may possibly be due to some post-transcriptional effect of TP
Pre-treatment with act.D, an inhibitor of transcription, caused a decrease in renal PRL-induced ODC mRNA to about 40% that of its level (Fig. 4), and enzyme activity was totally suppressed. This indicates that transcription is at least partially essential for the action of PRL on ODC. Actinomycin D has been reported to cause superinduction of ODC in certain systems (Wallon et al., 1990); however, no such effect was observed in the experimental model used here.

In kidney and liver, treatment with PRL induced ODC activity and increased mRNA levels. Treatment with the protein synthesis inhibitor cycloheximide caused a complete decline in ODC enzyme activity in both these organs (Tables 5 and 6), indicating that de novo protein synthesis is essential for the action of PRL on ODC. The ODC mRNA levels in both these organs were seen to increase slightly upon treatment with cycloheximide plus PRL (Figs. 5 and 6). This is likely to be due to the block in translation.

The signal transduction mechanisms linking prolactin receptors to macromolecular synthesis are poorly understood. Evidence has suggested that the mitogenic signalling enzyme protein kinase C (PKC) is involved in mediating PRL action in several target tissues, including rat liver. The pathway can be described briefly as follows: hormone or mitogen binding to its receptor stimulates the formation of diacylglycerol and inositol
triphosphate from Phosphatidyl inositol bisphosphate via the action of a G protein and the enzyme phospholipase C. Protein kinase C, in the presence of diacylglycerol, causes activation of particular proteins by phosphorylation, leading to the cellular response. The inositol triphosphate formed causes the release of calcium ions from the endoplasmic reticulum, also adding to the cellular response. The inhibitors used in this study, quercetin and quinacrine, inhibit the enzymes protein kinase C and phospholipase C, respectively.

It has been reported that in various tissues, PRL stimulates PKC activity. In cultured mouse mammary tissues, one of the earliest actions of PRL is the stimulation of ODC activity. The PKC inhibitor gossypol has been clearly shown to abolish the stimulatory effect of PRL on ODC activity (Etindi and Rillema, 1987), as well as its effects on lipid synthesis and RNA and casein synthesis. Therefore it is likely that at least some of the actions of PRL may involve protein kinase C.

In this study, quercetin, a PKC inhibitor known to have a minimal effect on RNA synthesis, was used to assess the effects of PKC inhibition on PRL-induced ODC activity in the kidney and liver of male adult rats. It was seen that at 6h after injection, 100 μM quercetin almost completely blocked the induction of ODC activity by PRL in the kidney (Table 7). In the liver, the same dose of quercetin had an even more dramatic inhibitory effect on ODC activity. This indicated that in kidney and liver, the stimulation of ODC activity by PRL is likely to be via
stimulation of the protein kinase C pathway.

In Nb2 node lymphoma cells, a cell line dependent on externally added PRL for growth, PRL-stimulated ODC activity was inhibited 95% by addition of 100 μM quercetin to the culture. The PKC inhibitors, polymyxin B and tamoxifen also inhibit ODC induction and proliferation in Nb2 node lymphoma cells.

Protein kinase C is known to be activated by diacylglycerols. The latter can be generated by the action of phospholipase C on phospholipids. Crowe et al. (1991) showed that in young male rats, PRL stimulated a dose-dependent induction of hepatic c-myc, ODC and β-actin mRNAs at 60 minutes after PRL administration. A dose of 22 mg/kg body weight of PRL increased diacylglycerol levels, followed by an increase in PKC levels.

In rat hepatocytes, it has been shown that PRL-stimulated alterations in PKC activity are preceded by enhanced diacylglycerol generation. In Nb2 lymphoma cells, quinacrine has been shown to inhibit PRL-stimulated ODC activity by almost 95%. Similarly, in mouse mammary gland explants, quinacrine inhibits ODC induction stimulated by the addition of either phospholipase A2 or C to the culture.

In this study, an attempt was made to further confirm the possible action of PRL via the protein kinase C pathway. The phospholipase A2 and C inhibitor, quinacrine, was administered in combination with PRL, to study the effect, if any. As shown in
Tables 9 and 10, the inhibitory effect of quinacrine on PRL-induced ODC activity was more pronounced in the liver than in the kidney. It is possible that in the latter, stimulation of PKC may be only one of the ways in which PRL affects ODC activity.

As mentioned in the introduction, a number of effects of prolactin have been linked to the stimulation of diacylglycerol formation and protein kinase C action. These include hepatotrophic responses stimulated by prolactin, and stimulation of ODC activity and mitogenesis in the Nb2 node lymphoma cell line. The results show that inhibition of phospholipase C and/or protein kinase C adversely affects the induction of ODC activity in the kidney and liver by PRL in vivo. The effect of PRL in enhancing ODC mRNA expression was seen to be more enhanced in the absence of testosterone, i.e. in castrated rats. The expression of PRL receptors have been shown to be regulated by testosterone in liver and kidney of male rats. It has been shown that PRL-binding activity in the kidney is decreased by testosterone treatment. In male rats, sexual maturation caused a decrease in hepatic PRL receptors, and these were restored upon castration. The induction of ODC activity and mRNA expression by PRL was seen to be dependent on both transcription and translation to various extents. Thus, PRL seems to play a role in regulating ornithine decarboxylase in the liver and kidney of rat, and the mechanism whereby it enhances ODC activity and expression seems to be via the formation of diacylglycerol and activation of protein kinase C, as inhibitors of DAG formation and of PKC prevented the stimulation of ODC by prolactin.
TABLE 1
TIME COURSE OF PROLACTIN ACTION ON ODC ENZYME ACTIVITY
IN KIDNEY OF ADULT MALE RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC specific activity (pinoles $^{14}$CO$_2$/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>2161 $\pm$ 49 (4)</td>
</tr>
<tr>
<td>2. PRL (1h)</td>
<td>2819 $\pm$ 161 (4)</td>
</tr>
<tr>
<td>3. PRL (2h)</td>
<td>3020 $\pm$ 95 (4)</td>
</tr>
<tr>
<td>4. PRL (4h)</td>
<td>9815 $\pm$ 131* (4)</td>
</tr>
<tr>
<td>5. PRL (6h)</td>
<td>10233 $\pm$ 225* (4)</td>
</tr>
<tr>
<td>6. Cas + Saline</td>
<td>5600 $\pm$ 205 (4)</td>
</tr>
<tr>
<td>7. Cas + PRL (6h)</td>
<td>6882 $\pm$ 116** (4)</td>
</tr>
</tbody>
</table>

Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200µl of vehicle. Animals were sacrificed at the indicated times after injection and the ODC activity was estimated. Controls received 200µl of vehicle alone.

Results are expressed as Mean $\pm$ S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - $p< 0.001$ as compared to group 1.

** - $p< 0.001$ as compared to group 6.
TABLE 2
TIME COURSE OF PROLACTIN ACTION ON LIVER ODC ACTIVITY IN ADULT MALE RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC specific activity (pmoles $^{14}$CO$_2$/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>1502 ± 47 (4)</td>
</tr>
<tr>
<td>2. PRL (1h)</td>
<td>1666 ± 24 (4)</td>
</tr>
<tr>
<td>3. PRL (2h)</td>
<td>1818 ± 140 (4)</td>
</tr>
<tr>
<td>4. PRL (4h)</td>
<td>2403 ± 80* (4)</td>
</tr>
<tr>
<td>5. PRL (6h)</td>
<td>2238 ± 97* (4)</td>
</tr>
</tbody>
</table>

Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Animals were sacrificed at the indicated times after injection and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - p< 0.001 as compared to group 1.
TABLE 3
EFFECT OF PROLACTIN ON KIDNEY ODC ACTIVITY OF CASTRATED RATS
TREATED WITH TESTOSTERONE PROPIONATE

Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Testosterone propionate was injected subcutaneously in 200μl of sesame oil at a dose of 350 μg/kg body weight. Animals were sacrificed at the indicated times after injection and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - p< 0.001 as compared to group 1
** - p< 0.001 as compared to group 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC specific activity (pmoles ¹⁴CO₂/ h/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>5794 ± 74 (4)</td>
</tr>
<tr>
<td>2. TP (6h)</td>
<td>8881 ± 277* (4)</td>
</tr>
<tr>
<td>3. PRL (6h)</td>
<td>6882 ± 203* (4)</td>
</tr>
<tr>
<td>4. TP + PRL (6h)</td>
<td>12381 ± 180 ** (4)</td>
</tr>
</tbody>
</table>
TABLE 4
EFFECT OF PRE-TREATMENT WITH ACTINOMYCIN D ON KIDNEY ODC ACTIVITY IN PRL-TREATED CASTRATED RATS

Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Actinomycin D was dissolved in ethanol: DMSO (1:1) and injected intraperitoneally 1h prior to prolactin at a dose of 6 mg/kg body weight. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - p< 0.001 as compared to group 1
** - p< 0.001 as compared to group 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC specific activity (pmoles $^{14}$CO₂/ h/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>5794 ± 74 (3)</td>
</tr>
<tr>
<td>2. PRL (6h)</td>
<td>9048 ± 99* (3)</td>
</tr>
<tr>
<td>3. Act. D (1h) + PRL(6h)</td>
<td>1146 ± 68** (3)</td>
</tr>
</tbody>
</table>
Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Cycloheximide was dissolved in saline and injected intraperitoneally 1h before sacrifice at a dose of 8 mg/kg body weight. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone. Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - p< 0.001 as compared to group 1

** - p< 0.001 as compared to group 2

TABLE 5
EFFECT OF TREATMENT WITH CYCLOHEXIMIDE ON KIDNEY ODC ACTIVITY IN PRL-TREATED CASTRATED RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC specific activity (pmoles ¹⁴CO₂/ h/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>5794 ± 74 (3)</td>
</tr>
<tr>
<td>2. PRL</td>
<td>7848 ± 259* (3)</td>
</tr>
<tr>
<td>3. CHX + PRL</td>
<td>2993 ± 60 (3)</td>
</tr>
</tbody>
</table>
TABLE 6
EFFECT OF TREATMENT WITH CYCLOHEXIMIDE ON LIVER
ODC ACTIVITY IN PRL-TREATED CASTRATED RATS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC specific activity (pmoles $^{14}$CO$_2$/ h/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>1917 ± 28 (4)</td>
</tr>
<tr>
<td>2. PRL</td>
<td>3041 ± 111* (4)</td>
</tr>
<tr>
<td>3. CHX + PRL</td>
<td>2065 - 72 (4)</td>
</tr>
</tbody>
</table>

Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Cycloheximide was dissolved in saline and injected intraperitoneally 1h before sacrifice at a dose of 8 mg/kg body weight. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - p< 0.001 as compared to group 1.
** - p< 0.005 as compared to group 2.
TABLE 7
EFFECT OF QUERCETIN, A PROTEIN KINASE C INHIBITOR, ON KIDNEY ODC ACTIVITY IN PRL-TREATED CASTRATED RATS

Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quercetin was dissolved in ethanol and injected intraperitoneally at a dose of 300 μmoles/kg body weight 15 minutes prior to injection of prolactin. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.
* - p< 0.001 as compared to group 1
** - p< 0.001 as compared to group 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ODC specific activity (pmoles 14CO₂/ h/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline</td>
<td>5600 ± 205 (4)</td>
</tr>
<tr>
<td>2. PRL</td>
<td>10800 ± 124* (4)</td>
</tr>
<tr>
<td>3. Quercetin + PRL</td>
<td>6022 ± 218** (4)</td>
</tr>
</tbody>
</table>
TABLE 8
EFFECT OF QUERCETIN, A PROTEIN KINASE C INHIBITOR ON PRL STIMULATION OF ODC LEVELS IN RAT LIVER

Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quercetin was dissolved in ethanol and injected intraperitoneally at a dose of 300 μmoles/kg body weight 15 minutes prior to injection of prolactin. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone. Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

*  -  p< 0.001 as compared to group 1.
**  -  p< 0.001 as compared to group 2.

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<tbody>
<tr>
<td>1. Saline</td>
<td>1917 ± 28 (3)</td>
</tr>
<tr>
<td>2. PRL (6 h)</td>
<td>3041 ± 112* (3)</td>
</tr>
<tr>
<td>3. Quercetin + PRL</td>
<td>1600 ± 63** (3)</td>
</tr>
</tbody>
</table>
TABLE 9
EFFECT OF QUINACRINE, A PHOSPHOLIPASE C INHIBITOR, ON KIDNEY ODC ACTIVITY IN PRL-TREATED CASTRATED RATS

Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quinacrine was dissolved in ethanol and injected intraperitoneally at a dose of 200 μmoles/kg body weight 15 minutes prior to prolactin. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* p< 0.001 as compared to group 1.
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<td>2. PRL</td>
<td>10800 ± 124* (4)</td>
</tr>
<tr>
<td>3. Quinacrine + PRL</td>
<td>7812 ± 146 (4)</td>
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</tbody>
</table>

* p< 0.001 as compared to group 1.
** p< 0.001 as compared to group 2.
TABLE 10
EFFECT OF QUINACRINE, A PHOSPHOLIPASE C INHIBITOR ON PRL STIMULATION OF ODC IN RAT LIVER

Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quinacrine was dissolved in ethanol and injected intraperitoneally at a dose of 200 μmoles/kg body weight 15 minutes prior to prolactin. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - P< 0.001 as compared to group 1.
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<td>3041 ± 111* (3)</td>
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<td>3. Quinacrine + PRL</td>
<td>2021 ± 117** (3)</td>
</tr>
</tbody>
</table>

*Animals were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quinacrine was dissolved in ethanol and injected intraperitoneally at a dose of 200 μmoles/kg body weight 15 minutes prior to prolactin. Animals were sacrificed 6h after injection of prolactin and the ODC activity was estimated. Controls received 200μl of vehicle alone.

Results are expressed as Mean ± S.E.M. of 3-4 determinations from 4-5 animals in each group.

* - P< 0.001 as compared to group 1.
* - p< 0.005 as compared to group 2.
Fig. 1. Time course of prolactin action on ODC expression in the kidney of rat.

Adult rats of 300-350g weight were used. The rats in the castrated group (Lane 5) were castrated via the scrotal route 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Controls received 200μl of vehicle alone. Animals were sacrificed at the indicated times after injection. RNA isolation and northern analysis were carried out as described in Chapter 2.

A. Autoradiogram of northern blot showing ODC expression of PRL-treated kidney.

B. Methylene blue stained ribosomal RNA (28S and 18S) of the blot demonstrating equal loading of the samples.

C. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 1

(A) Gel electrophoresis showing bands at 2.6 kb and 2.2 kb.

(B) Gel showing bands at 28S and 18S.

(C) Bar graph showing relative expression (% of maximum) over time:
- Control
- PRL 1h
- PRL 2h
- PRL 6h
- Cas+PRL 6h
Fig. 2. Time course of prolactin action on ODC expression in the liver of rat.

Adult rats of 300-350g weight were used. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Controls received 200μl of vehicle alone. Animals were sacrificed at the indicated times after injection. Liver RNA isolation and northern analysis were carried out as described in Chapter 2.

A. Autoradiogram of northern blot showing ODC expression of PRL-treated liver.

B. Methylene blue stained ribosomal RNA (28S and 18S) of the blot demonstrating equal loading of the samples.

C. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 3. Effect of prolactin on kidney ODC expression in rats treated with testosterone propionate.

Adult rats of 300-350g weight were castrated via the scrotal route 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Testosterone propionate (TP) was injected subcutaneously in 200μl of sesame oil at a dose of 350 Mg/kg body weight. Animals were sacrificed 6h after injection. RNA isolation and dot blot analysis were carried out as described in Chapter 2.

A. Autoradiogram of northern blot showing the effect of PRL on ODC expression of TP-treated Kidney.
B. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 3
Fig. 4. Effect of pre-treatment with **actinomycin D** on PRL-induction of ODC expression in kidney of rat.

Adult rats of 300-350g body weight were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. **Actinomycin D** was dissolved in ethanol:DMSO (1:1) and injected intraperitoneally 1h prior to prolactin at a dose of 6 mg/kg body weight. Controls received 200ml of vehicle. Animals were sacrificed 6h after injection of prolactin. Kidney RNA isolation and northern blot analysis were carried out as described in Chapter 2.

A. Autoradiogram of northern blot showing the effect of pre-treatment with Actinomycin D on ODC expression of PRL-treated liver.

B. **Methylene** blue stained ribosomal RNA (28S and 18S) of the blot demonstrating equal loading of the samples.

C. **Densitometric** data of A expressed as relative percent of maximal expression.
Fig. 4
Animals of 300-350g body weight were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Cycloheximide was dissolved in ethanol and injected intraperitoneally 1h before sacrifice at a dose of 8 mg/kg body weight. Controls received 200μl of vehicle alone. Animals were sacrificed 6h after injection of prolactin. RNA isolation and northern analysis were carried out as described in Chapter 2.

A. Autoradiogram of northern blot showing the effect of treatment with cycloheximide on ODC expression of PRL-treated kidney.

B. Methylene blue stained ribosomal RNA (28S and 18S) of the blot demonstrating equal loading of the samples.

C. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 5
Animals of 300-350g body weight were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Cycloheximide was dissolved in ethanol and injected intraperitoneally 1h before sacrifice at a dose of 8 mg/kg body weight. Controls received 200μl of vehicle alone. Animals were sacrificed 6h after injection of prolactin. RNA isolation and northern analysis were carried out as described in Chapter 2.

A. Autoradiogram of northern blot showing the effect of treatment with cycloheximide on ODC expression of PRL-treated liver.

B. Methylene blue stained ribosomal RNA (28S and 18S) of the blot demonstrating equal loading of the samples.

C. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 6

(A) Control, PRL, CHX + PRL

(B) 28 S, 18 S

(C) Relative expression (% of maximum)
Fig. 7. Effect of quercetin, a protein kinase C inhibitor, on kidney ODC expression in PRL-treated rats.

Adult rats of 300-350g body weight were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200µl of vehicle. Quercetin was dissolved in ethanol and injected intraperitoneally at a dose of 300 µmoles/kg body weight 15 minutes prior to injection of prolactin. Controls received 200µl of vehicle alone. Animals were sacrificed 6h after injection of prolactin. RNA isolation and dot blot analysis were carried out as described in Chapter 2.

A. Autoradiogram of northern blot showing the effect of treatment with quercetin on ODC expression of PRL-treated kidney.

B. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 7
Fig. 8. Effect of quercetin, a protein kinase inhibitor, on liver ODC expression in PRL-treated rats.

Adult rats of 300-350g body weight were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quercetin was dissolved in ethanol and injected intraperitoneally at a dose of 300 μmoles/kg body weight 15 minutes prior to injection of prolactin. Controls received 200μl of vehicle alone. Animals were sacrificed 6h after injection of prolactin. RNA isolation and dot blot analysis were carried out as described in Chapter 2.

A. Autoradiogram of dot blot showing the effect of treatment with quercetin on ODC expression of PRL-treated liver.

B. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 8

(A) 

(B) 

Relative expression (% of maximum)
Fig. 9. Effect of quinacrine, phospholipase C inhibitor, on kidney ODC expression in PRL-treated rats.

Adult rats of 300-350g body weight were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quinacrine was dissolved in ethanol and injected intraperitoneally at a dose of 200 μmoles/kg body weight 15 minutes prior to prolactin. Controls received 200μl of vehicle alone. Animals were sacrificed 6h after injection of prolactin. RNA isolation and dot blot analysis were carried out as described in Chapter 2.

A. Autoradiogram of dot blot showing the effect of treatment with quinacrine on ODC expression of PRL-treated kidney.

B. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 9
Fig. 10. Effect of quinacrine, phospholipase C inhibitor, on liver ODC expression in PRL-treated rats.

Adult rats of 300-350g body weight were castrated 4 days prior to use. Prolactin was dissolved in 0.9% sodium chloride/0.1N NaOH and injected intraperitoneally at a dose of 5.5 mg/kg body weight in 200μl of vehicle. Quinacrine was dissolved in ethanol and injected intraperitoneally at a dose of 200 μmoles/kg body weight 15 minutes prior to prolactin. Controls received 200μl of vehicle alone. Animals were sacrificed 6h after injection of prolactin. RNA isolation and dot blot analysis were carried out as described in Chapter 2.

A. Autoradiogram of dot blot showing the effect of treatment with quinacrine on ODC expression of PRL-treated liver.

B. Densitometric data of A expressed as relative percent of maximal expression.
Fig. 10