Biosynthesis and Degradation of Prolactin in the Rat Anterior Pituitary Gland

TIME COURSE OF INCORPORATION OF LABEL *IN VITRO* AND EVIDENCE FOR RAPID DEGRADATION

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Biosynthesis of prolactin was studied in anterior pituitary glands from female rats, incubated *in vitro*. In this system [^3H]leucine was incorporated into pituitary proteins, including somatotropin (growth hormone) and prolactin. The rate of uptake of label into prolactin (and to a lesser extent into total protein) slowed considerably during the first 2 h of incubation, although the rate of uptake into somatotropin was constant for 8 h. The most probable explanation for this apparent decrease in the rate of prolactin synthesis is degradation of prolactin in the gland. Degradation of this hormone was also demonstrated by incubating prelabelled pituitaries in unlabelled medium and following the content of labelled prolactin, and by studying the hormonal content of pituitary glands (by radioimmunoassay) before and after incubation. Degradation of prolactin appears to be much more rapid than that of somatotropin, and may represent a physiological mechanism whereby over-accumulation of prolactin is prevented when secretion of the hormone has been rapidly switched off.

Synthesis of prolactin and somatotropin (growth hormone) by the anterior lobe of the rat pituitary gland can be readily studied *in vitro*. Thus, when anterior pituitary lobes or cells are incubated *in vitro* in a buffered salts medium containing glucose and a labelled amino acid, they incorporate label into proteins, including prolactin and somatotropin. The amount of label incorporated can be measured after separation of these hormones from other proteins by electrophoresis or immunoprecipitation.

Such an approach can be used to investigate the actions of various substances on prolactin synthesis *in vitro*, and has been so used by several authors (e.g. MacLeod & Abad, 1968; Labrie *et al.*, 1973; Shenai & Wallis, 1974, 1975). In the course of studies of this kind we carried out a detailed investigation of the nature of prolactin biosynthesis *in vitro*, which suggested that the process is more complex than is usually recognized. In particular, degradation of prolactin within the pituitary gland must be considered in addition to biosynthesis and secretion; such degradation seems to be considerably more important for prolactin than for somatotropin. Corresponding complexity in the intracellular transport and packaging of prolactin has also been demonstrated by morphological studies (Farquhar, 1971; Farquhar *et al.*, 1978). This paper describes several experiments that suggest the importance of prolactin degradation.

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Materials and Methods

Materials

Rat prolactin (NIAMDD-rat prolactin-RP-1; 11 i.u./mg), rat somatotropin (NIAMDD-rat GH-B-1; 0.9 i.u./mg) and materials for radioimmunoassay of these hormones were generously provided by Dr. A. Parlow and the Rat Pituitary Hormone Program of the National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, MD, U.S.A.

L-[4,5- ^3H]Leucine (specific radioactivity 50–60 Ci/mmol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Tissue culture medium 199 was from Flow Laboratories (Irvine, Scotland, U.K.).

Animals

Pituitary glands were obtained from virgin female rats (8–10 weeks old) of the albino Sprague–Dawley strain. These animals were obtained from the laboratory colony and were fed *ad lib*.

Incubation of anterior pituitary glands

Rats were killed by cervical dislocation. The pituitary glands were removed immediately, anterior and posterior lobes were separated, and the former were placed in sterile Krebs–Ringer bicarbonate medium [118 mM-NaCl, 4.7 mM-KCl, 2.5 mM-CaCl₂, 1.2 mM-MgSO₄, 25 mM-NaHCO₃, 1.2 mM-KH₂PO₄, 20 mg of Phenol Red/litre, 35 mg of penicillin G/litre and 100 mg of streptomycin sulphate/litre, equilibrated with CO₂/air (1:19), giving a final pH of about

7.4] at 20–25°C. Rats were always killed between 09:00 and 10:00h, in order to minimize variations in the state of the gland due to the oestrous cycle or diurnal rhythm (Gay *et al.*, 1970; Neill, 1970).

Glands were preincubated for 15 min in Krebs–Ringer bicarbonate medium, and then bisected into 'hemipituitaries'. These were placed into sterile polystyrene incubation tubes (75 mm × 12 mm), and incubated in Krebs–Ringer bicarbonate medium containing 11.2 mM-glucose and (for the biosynthesis experiments) L-[³H]leucine (5 μ Ci/ml). Normally, two hemipituitaries were divided into four fragments and incubated (in one tube) in 0.5 ml of medium. Incubation was carried out at 37°C in a humidified atmosphere containing CO₂/air (1:19). After incubation, glands were removed from the medium, blotted and weighed on a torsion balance. They were then homogenized in 0.5 ml of 0.1 M-Tris adjusted to pH 9.5 with acetic acid. Media and homogenates were each dialysed overnight at 4°C against L-leucine (1 mg/ml) to remove the remaining free [³H]leucine.

Measurement of incorporation of [³H]leucine into protein

Samples of the incubation media and pituitary homogenates were analysed to measure incorporation of [³H]leucine into protein. Dialysed media and homogenates (50 μ l), plus about 100 μ g of bovine serum albumin in each case, were precipitated with 2 ml of 5% (w/v) trichloroacetic acid containing 1 mg of unlabelled L-leucine/ml. After 4 h, precipitates were collected by filtration on glass-fibre discs, washed with more trichloroacetic acid, dried overnight at 37°C and submitted to scintillation counting in toluene containing 0.35% 2,5-diphenyloxazole and 0.005% 1,4-bis-(5-phenyloxazol-2-yl)benzene dimethyl ester. Counting efficiency (in a Beckman LS233 scintillation counter) was about 35%.

Measurement of incorporation of [³H]leucine into prolactin and somatotropin

Incorporation of label into prolactin and (in some cases) somatotropin was measured by fractionating the pituitary homogenate or incubation medium by polyacrylamide-gel electrophoresis and then solubilizing the appropriate hormone-containing band and submitting it to scintillation counting. Polyacrylamide gels were made as described by Davis (1964), but sample and spacer gels were not used. Final acrylamide concentration was 8.0% (w/v), with 2.5% (w/w) cross-linker (Wallis *et al.*, 1978). Duplicate 50 μ l portions of the pituitary homogenates and 100 μ l portions of media were submitted to electrophoresis. After electrophoresis, gels were stained in 0.005% Amido Black in 7.5% (v/v) acetic acid for 3–4 h, and then destained in 7.5% acetic acid

containing 0.1% leucine. The prolactin band (located by comparison with gels on which marker prolactin had been run; see also Jones *et al.*, 1965) was excised, solubilized with 30% H₂O₂ (60°C, overnight) and submitted to scintillation counting in a water-miscible scintillator (0.5% 2,5-diphenyloxazole/70% toluene/30% Triton X-100, w/v/v). In some cases the entire polyacrylamide gel was sliced (0.5 cm slices), solubilized and counted for radioactivity. Counting efficiency was 20–25%.

Scanning densitometry of stained polyacrylamide gels was carried out by a Joyce–Loebl 'chromoscan' or a Unicam SP.1800 spectrophotometer with scanning attachment.

Prelabelling experiments for study of prolactin degradation

In order to study the degradation of newly synthesized prolactin in the pituitary gland, hemipituitaries were removed from female rats and preincubated as described above. Each pair of hemipituitaries was then labelled by incubating, as described above, for 4 h in 0.8 ml of Krebs–Ringer bicarbonate medium containing 20 μ Ci of [³H]leucine/ml. One of each pair of glands was removed, weighed and homogenized, and the other was incubated further in 0.4 ml of fresh medium containing unlabelled 3 μ M-L-leucine. After 1, 2 or 3 h of such incubation in unlabelled medium these hemipituitaries were also homogenized. The medium and homogenates were analysed (without prior dialysis) for incorporation of label into protein, prolactin and somatotropin as described above.

Radioimmunoassay of rat prolactin and somatotropin

The concentrations of prolactin and somatotropin in incubation media and homogenates of pituitary glands were determined by radioimmunoassay, using reagents provided by the National Institute of Arthritis, Metabolism and Digestive Diseases (Bethesda, MD, U.S.A.). The procedures were based on those described in the instructions accompanying the reagents, except that precipitation of antibody–hormone complexes was carried out with poly(ethylene glycol), as described by Desbuquois & Aurbach (1971).

Results

Incorporation of L-[³H]leucine into pituitary protein

When anterior pituitaries from female rats were incubated *in vitro* with [³H]leucine, label was incorporated into protein; a time course is shown in Fig. 1. Accumulation of label into protein was rapid in the first hour of incubation but then slowed to about half the previous rate, and remained approximately linear for a further 6 h. The result is somewhat

different from that seen with male rat pituitaries (Betteridge & Wallis, 1973), where incorporation into pituitary protein proceeds in a linear fashion throughout the incubation.

Incorporation of L-[³H]leucine into prolactin and somatotropin

The synthesis of prolactin and somatotropin by anterior pituitary glands incubated *in vitro* with [³H]leucine was demonstrated by subjecting samples of the homogenized glands and of the incubation media to polyacrylamide-gel electrophoresis, performing densitometric scanning, solubilizing slices of the gels and measuring the radioactivity in these slices. After 1 h there was a significant L-[³H]leucine incorporation into somatotropin and prolactin in the pituitary gland, and this increased after 4 and 8 h (Fig. 2a). However, the total quantity of (unlabelled) prolactin (measured by densitometry) decreased during the incubation.

Labelled prolactin was detectable in the incubation medium after 1 h, and the quantity increased rapidly during further incubation (Fig. 2b). Labelled

somatotropin appeared in the medium much more slowly. The rate at which unlabelled prolactin appeared in the medium (estimated by densitometry) was much slower, and could not account for the rapid disappearance of this material from the gland.

Accumulation of labelled prolactin within the anterior pituitary is very rapid in the first 30 min, but then slows considerably (Fig. 3). Part of this slowing can be accounted for by release of labelled hormone into the medium (which begins after a lag of about 40 min), but when the total accumulation of label into prolactin (in gland and medium) is considered, the marked lowering of the rate of 'apparent incorporation' is still seen (Fig. 3). Accumulation of labelled somatotropin (medium and pituitary gland combined) was virtually linear during the 8 h incubation period (Fig. 4), and contrasted markedly with the corresponding labelling pattern for prolactin.

Further investigation of the time course of incorporation of L-[³H]leucine into prolactin

The non-linear nature of the time course for incorporation of label into prolactin seen in Figs. 3 and 4 was surprising, particularly in the light of the corresponding linearity seen for somatotropin (Fig. 4). Slowing of prolactin synthesis during incubation could result from shortage of some essential nutrients in the simple medium used. However, when a tissue culture medium (199) containing a range of nutrients (Morgan *et al.*, 1950) was used as an alternative incubation medium, the time course of apparent prolactin synthesis again showed a considerable lowering of the rate of synthesis after the first hour of incubation (Fig. 5).

L-[³H]Leucine was not depleted during the course of incubation; at least 90% of that added was still present in the medium at the end of the incubation (as judged by high-voltage electrophoresis of samples of medium).

Increasing the period of preincubation of the hemipituitaries to 1 h (in Krebs-Ringer bicarbonate medium containing 11.2 mm-glucose), with change of medium and addition of [³H]leucine after 1 h, had no effect on the subsequent time course of incorporation of [³H]leucine into prolactin; the apparent slowing of prolactin synthesis during the first hour of incubation was still observed. The changes occurring during this period were thus not due to changes in the anterior pituitary during the first hour of incubation, since such changes would have occurred in this experiment before the study of leucine incorporation was commenced.

Tissue viability during incubation

The nature of the time course for incorporation of [³H]leucine into pituitary protein, somatotropin and prolactin suggested that the hemipituitaries were viable during the course of the 8 h incubations. Two

Fig. 1. *Incorporation of L-[³H]leucine into pituitary proteins*

Anterior pituitaries from female rats were incubated in Krebs-Ringer bicarbonate medium containing 11.2 mm-glucose and [³H]leucine (5 μ Ci/ml). At various times glands were removed, weighed and homogenized. Media and homogenates were analysed for incorporation of [³H]leucine into trichloroacetic acid-insoluble material, and the results were combined for each incubation to show total incorporation of [³H]leucine into pituitary protein. Each point is the mean of at least three separate determinations.

Fig. 2. Polyacrylamide-gel electrophoresis of proteins synthesized *in vitro* by pituitary glands from female rats. Anterior pituitaries from female rats were incubated in Krebs-Ringer bicarbonate medium containing 11.2 mM-glucose and [³H]leucine (5 μ Ci/ml) for 1, 4 or 8 h. The tissue was weighed and homogenized and samples of the homogenates (a) and media (b) were submitted to polyacrylamide-gel electrophoresis. Gels were stained, destained, cut into 5 mm slices and treated for scintillation counting. Positions of somatotropin (S) and prolactin (P) are indicated.

Fig. 3. Incorporation of $[^3\text{H}]$ leucine into rat prolactin Anterior pituitaries from female rats were incubated in Krebs–Ringer bicarbonate medium containing 11.2mM-glucose and $[^3\text{H}]$ leucine ($5\mu\text{Ci/ml}$). At various times, glands were removed from the incubation medium, weighed and homogenized. Samples of the media and pituitary homogenates were submitted to polyacrylamide-gel electrophoresis and the prolactin-containing band was excised from each gel, solubilized and submitted to scintillation counting. Incorporation of label into rat prolactin retained in the gland (●) and released into the medium was measured (■) and the total incorporation is also shown (▲). Each point is the mean of at least three determinations.

Fig. 4. Incorporation of L- $[^3\text{H}]$ leucine into rat somatotropin and prolactin

Anterior pituitaries from female rats were incubated for various times in Krebs–Ringer bicarbonate medium containing 11.2mM-glucose and $[^3\text{H}]$ leucine ($5\mu\text{Ci/ml}$). Glands were removed from the medium after incubation, weighed and homogenized. Samples of the medium and homogenates were submitted to polyacrylamide-gel electrophoresis, and the prolactin- and somatotropin-containing bands were excised from each gel, solubilized and submitted to scintillation counting. Total incorporation of label (homogenate and medium combined for each sample) into rat prolactin (■) and somatotropin (▲) is shown. Each experimental point is the mean of at least three separate determinations.

further studies were carried out to establish tissue viability at the end of the incubation period.

Extended preincubations of hemipituitaries in Krebs–Ringer bicarbonate/glucose medium were carried out (for 4, 5, 6 and 7 h in addition to the usual 15 min). After preincubation the medium was changed to one containing $[^3\text{H}]$ leucine, and incubation was continued for a further 1 h. In no case did the amount of $[^3\text{H}]$ leucine incorporated into protein or prolactin differ significantly from that incorporated in a control pituitary preincubated for only 15 min, and then incubated with $[^3\text{H}]$ leucine for 1 h (Table 1). Thus the apparent rate of incorporation of $[^3\text{H}]$ leucine into protein or prolactin was the same whether glands had been incubated *in vitro* for 0.25 or 7.25 h.

Pituitary tissue that had been incubated *in vitro* for 8 h was also examined for ultrastructural changes, by electron microscopy. Secretory granules, Golgi

apparatus, endoplasmic reticulum and mitochondria had all remained intact and apparently unchanged during the incubation, though small changes in the nucleus were detected in some cases. Pelletier *et al.* (1972) also showed that rat anterior pituitaries that had been incubated *in vitro* for 6 h, in a medium very similar to that used here, showed no obvious ultrastructural deterioration under the electron microscope.

Degradation of newly synthesized prolactin and somatotropin

The non-linear time course seen for incorporation of $[^3\text{H}]$ leucine into prolactin could be due to rapid degradation of newly synthesized prolactin (see the Discussion section). The possibility that such newly synthesized prolactin is rapidly degraded was studied by prelabelling paired hemipituitaries with $[^3\text{H}]$ -

Fig. 5. Incorporation of L-[³H]leucine into rat prolactin by pituitaries incubated in tissue culture medium 199

Anterior pituitaries from female rats were incubated for various times in tissue culture medium 199 containing [³H]leucine (5 μ Ci/ml). Glands were removed from medium after incubation, weighed and homogenized. Portions of media and homogenates were analysed by polyacrylamide-gel electrophoresis and the prolactin-containing band was excised from each gel, solubilized and submitted to scintillation counting. Incorporation of label into prolactin in the media (■), homogenates (●) and the two combined (▲) is shown. Each experimental point is the mean of three separate determinations.

leucine and then extracting one of each pair directly, the other being subjected to further incubation in unlabelled medium.

The results of such an experiment are shown in Table 2. After 3 h incubation in unlabelled medium the total content (gland plus medium) of labelled prolactin had been lowered to 67% of that in the unincubated (prelabelled) control. The content of labelled somatotropin was unchanged in this 3 h incubation period. It could not be determined to what extent incorporation of [³H]leucine occluded within the gland continued during the 3 h incubation in unlabelled medium, and the apparent degradation observed may therefore be an underestimate. However, the results do suggest that there is a fairly rapid degradation of prelabelled prolactin (but not somatotropin) within the incubated pituitary.

Disappearance of prolactin and somatotropin during incubation of pituitary glands in vitro, as measured by radioimmunoassay

In another attempt to assess the importance of degradation of prolactin and somatotropin during incubation of the pituitary, the content of these hormones was measured by radioimmunoassay, before and after incubation. Paired hemipituitaries from female rats were again used, one of each pair being extracted directly, while the other was incubated for 1, 2 or 4 h and then extracted.

Table 3 shows the results. The total prolactin content (gland plus medium) was lowered substantially during incubation, whereas that of somatotropin was only slightly (and non-significantly) lowered. Again it appears that there is substantial degradation of prolactin, but not of somatotropin, during incubation. Although continued synthesis of somatotropin and prolactin occurs during incubation (as shown by incorporation of [³H]leucine), net synthesis of these hormones probably cannot occur, since there are no precursor amino acids in the incubation medium.

Table 1. Effect of time of incubation on viability of anterior pituitary tissue

Anterior pituitaries from female rats were preincubated for 4, 5, 6 and 7 h in Krebs-Ringer bicarbonate medium containing 11.2 mM-glucose, in addition to the usual 0.25 h preincubation. [³H]Leucine (5 μ Ci/ml) was added for a further 1 h. Incorporation of [³H]leucine into prolactin and total protein during this final hour was measured. Results are shown as the means $\pm$ S.E.M. for three experiments. There are no results significantly different from control.

Period of preincubation (h)	...	0 (Control)	4	5	6	7
Incorporation into total protein (d.p.m./mg of pituitary)		1876 $\pm$ 113.6	1457 $\pm$ 131.6	1526 $\pm$ 101.3	1693 $\pm$ 96.3	2207 $\pm$ 121.6
Prolactin synthesis						
(a) d.p.m./mg of pituitary		562 $\pm$ 41.3	423 $\pm$ 80.3	479 $\pm$ 56.3	518 $\pm$ 61.3	679 $\pm$ 103.4
(b) Percentage of total incorporation into protein		30.0 $\pm$ 0.9	31.2 $\pm$ 0.5	31.4 $\pm$ 1.0	30.6 $\pm$ 0.9	30.7 $\pm$ 1.0

Table 2. *Degradation of prelabelled prolactin and somatotropin in incubated pituitary glands*

Paired hemipituitaries from female rats were incubated for 4 h in Krebs-Ringer bicarbonate medium containing 11.2 mM-glucose and 20 μ Ci of L-[3 H]leucine/ml. One hemipituitary of each pair was then homogenized and the second was incubated further in unlabelled medium (containing 11.2 mM-glucose and 3 μ M-L-leucine) for 1, 2 or 3 h, before homogenization. Incorporation of [3 H]leucine into prolactin and somatotropin was determined for the control hemipituitaries (not incubated in unlabelled medium) and experimental hemipituitaries (incubated in unlabelled medium; results based on label in gland plus incubation medium). Results are means $\pm$ S.E.M. for the numbers of observations indicated. Results from two separate experiments have been combined. Percentage recovery in experimental hemipituitaries was based on paired observations, and hence was not identical with percentage obtained from the pooled observations shown in the previous two columns.

Prolactin

Incubation time (h)	No. of observations	Radioactivity in prolactin		
		Percentage of total in protein		Recovery in experimental (percentage of control)
		Control	Experimental	
1	4	13.8 $\pm$ 1.0	12.8 $\pm$ 1.1	94 $\pm$ 8.5
2	5	13.4 $\pm$ 1.4	10.9 $\pm$ 1.5	84 $\pm$ 13.8
3	7	18.7 $\pm$ 2.0	12.3 $\pm$ 1.2*	67 $\pm$ 4.5**

Somatotropin

Incubation time (h)	No. of observations	Radioactivity in somatotropin		
		Percentage of total in protein		Recovery in experimental (percentage of control)
		Control	Experimental	
1	4	14.1 $\pm$ 1.3	13.1 $\pm$ 1.8	93 $\pm$ 10.9
2	5	15.1 $\pm$ 2.0	14.1 $\pm$ 1.5	99 $\pm$ 16.0
3	7	15.8 $\pm$ 1.7	14.9 $\pm$ 0.4	101 $\pm$ 11.5

* $P < 0.02$ (compared with corresponding control) (Student's t test).

** $P < 0.001$ (compared with 100%) (Student's t test).

Table 3. *Prolactin and somatotropin content of female rat pituitary glands before and after incubation*

Paired hemipituitaries were removed from female rats. One of each pair was extracted immediately and the other was incubated in Krebs-Ringer bicarbonate medium containing 11.2 mM-glucose, for 1, 2 or 4 h, and then extracted. Prolactin and somatotropin contents were determined by radioimmunoassay of unincubated (pituitary extract) and incubated (extract plus medium) hemipituitaries. Results are means $\pm$ S.E.M. for the numbers of observations indicated.

Prolactin

Incubation time (h)	No. of observations	Prolactin content (μ g/mg wet wt.)†		Recovery of prolactin after incubation (%)‡
		Unincubated hemipituitaries	Incubated hemipituitaries	
1	5	6.02 $\pm$ 0.66	4.72 $\pm$ 0.68	77.3 $\pm$ 5.4*
2	7	5.57 $\pm$ 0.93	3.64 $\pm$ 0.51	67.7 $\pm$ 5.3**
4	5	5.29 $\pm$ 0.84	3.74 $\pm$ 0.56	73.0 $\pm$ 7.8*

Somatotropin

Incubation time (h)	No. of observations	Somatotropin content (μ g/mg wet wt.)§		Recovery of somatotropin after incubation (%)‡
		Unincubated hemipituitaries	Incubated hemipituitaries	
1	5	82.1 $\pm$ 4.9	73.5 $\pm$ 6.2	88.3 $\pm$ 4.4
2	7	71.1 $\pm$ 5.3	70.5 $\pm$ 7.2	98.5 $\pm$ 6.4
4	5	76.7 $\pm$ 3.6	70.0 $\pm$ 2.4	88.2 $\pm$ 6.7

* $P < 0.05$ (compared with 100%; Student's t test)

** $P < 0.001$ (compared with 100%; Student's t test)

† In terms of rat prolactin standard: NIAMDD-rat prolactin-RP-1.

‡ Based on paired observations.

§ In terms of rat somatotropin standard: NIAMDD-rat growth hormone-RP-1.

Discussion

Hemipituitaries incubated *in vitro* incorporated L-[³H]leucine into pituitary protein, somatotropin and prolactin. There was a marked difference, however, between the time courses for uptake of label into somatotropin and prolactin (Fig. 4). That for somatotropin was linear with time [as has been seen previously with male rats (Betteridge & Wallis, 1973)], but that for prolactin showed a marked slowing during the first 2 h of incubation. The incorporation of L-[³H]leucine into total pituitary protein was linear in pituitaries from male rats (Betteridge & Wallis, 1973), but slowed during the first 2 h of incubation in pituitaries from female rats (Fig. 1), probably because of the much greater production of prolactin in the pituitaries of females.

The non-linear nature of the time courses for prolactin synthesis does not appear to be due to depletion of components of the medium, decreased tissue viability or alterations occurring in the pituitary gland during the first 1–2 h of incubation. Accumulation of a factor (possibly prolactin itself) in the incubation medium might cause a slowing of prolactin synthesis, but the time course of the process and the experiments shown in Table 1 do not accord well with such an explanation. [A direct action of prolactin on the pituitary gland would accord with observations that the pituitary contains 'receptors' for prolactin (Frantz & Sonnenschein, 1974) and that pituitary prolactin synthesis is lowered in rats carrying prolactin-secreting tumours (MacLeod & Abad, 1968).]

An alternative explanation for the non-linear time course for prolactin synthesis could be degradation of prolactin within the pituitary gland (degradation after secretion would not accord with the linear accumulation of labelled prolactin that occurs in the medium). This could apply if prolactin contained in mature (or immature) secretory granules was continuously being broken down (or processed) within the gland, while newly synthesized prolactin was not degraded in this fashion until it became incorporated into granules. The rate of appearance of labelled prolactin would then be rapid in the first hour of incubation and slow subsequently, not because the real rate of synthesis was lowered but because a good deal of what had been synthesized during the earlier part of the incubation had already been degraded or altered within the gland. Alteration of the hormone need not necessarily involve inactivation; indeed, activation of prolactin could be occurring, as suggested by Asawaroengchai & Nicoll (1977). Eventually a steady-state relationship would be achieved between synthesis and degradation, where accumulation of labelled protein continued at a linear rate, considerably lower than that observed at first.

In order to test whether degradation of prolactin could be demonstrated directly we prelabelled pituitary glands from female rats and followed the content of labelled prolactin and somatotropin during subsequent incubation in unlabelled medium. The content of labelled somatotropin was unchanged during a 3 h incubation, but that of prolactin was lowered by 33% (Table 2). Loss of prolactin was also followed by radioimmunoassay of the hormone contents of incubated or unincubated glands. Again there was a significant loss of prolactin but not of somatotropin (Table 3). Similar loss of prolactin was observed using densitometry to measure the hormone. In each of these experiments we cannot eliminate the possibility that synthesis of (labelled or unlabelled) hormones continued during the incubation, which would lead to an underestimate of the percentage degradation of prolactin. However, it is clear that prolactin is degraded relatively rapidly during incubation and that somatotropin is not, in agreement with the explanation given in the previous paragraph for the non-linear time course for incorporation of label into prolactin.

These experiments suggesting a degradation of prolactin within the pituitary gland accord with observations showing that in some circumstances degradation of prolactin granules can be demonstrated by electron microscopy, as a consequence of fusion of granules with lysosomes (crinophagy) (Farquhar, 1971; Farquhar *et al.*, 1978; Pasteels, 1972). Nicoll & Swearingen (1970) have also obtained evidence for a fairly rapid turnover of prolactin within the pituitary gland. Such degradation of prolactin, shown by both morphological and biochemical approaches, may represent a physiological mechanism for dealing with overproduction of the hormone under circumstances where secretion of the hormone has to be switched off rapidly (e.g. when the suckling young are removed from a lactating animal). It seems clear, however, that significant degradation is occurring under much less extreme conditions. The contrast with somatotropin is noteworthy; here physiological fluctuations of secretion are likely to be much less drastic, and degradation of the hormone is much less marked.

Our time course results do not agree completely with those of MacLeod & Abad (1968) and MacLeod & Lehmyer (1972), who found that the rate of prolactin secretion increased during 8 h of incubation of rat anterior pituitaries, and that the proportion of labelled prolactin secreted into the medium was greater than we have observed. The discrepancy could lie in the use of different extraction conditions. MacLeod and his colleagues extracted at pH 7.2, which is known to give less complete extraction than at pH 9.5 (Zanini & Giannattasio, 1972). Incomplete extraction would give an underestimate of the total amount of labelled prolactin formed, especially for

early time points when little hormone has been secreted. Hill *et al.* (1976) and Augustine & MacLeod (1975) used more effective extraction conditions and obtained values for the proportion of labelled prolactin secreted that agree well with ours. It should be noted that in longer-term cultures of pituitary glands, the rate of prolactin synthesis increases considerably; such an acceleration may be starting after 6–8 h in some of our experiments (e.g. Figs. 4 and 5).

Incubation *in vitro* of rat pituitary glands has been used widely for studies on the secretion and synthesis of prolactin. It is now clear that regulation of the quantity of prolactin within the gland is a complex process which may involve modulation of synthesis, secretion and degradation. All three processes must be taken into account when the action of various agents on prolactin production is considered.

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References

- Asawaroengchai, H. & Nicoll, C. S. (1977) *J. Endocrinol.* **73**, 301–308
- Augustine, E. C. & MacLeod, R. M. (1975) *Proc. Soc. Exp. Biol. Med.* **150**, 551–556
- Betteridge, A. & Wallis, M. (1973) *Biochem. J.* **134**, 1103–1113
- Davis, B. J. (1964) *Ann. N.Y. Acad. Sci.* **121**, 404–427
- Desbuquois, B. & Aurbach, G. D. (1971) *J. Clin. Endocrinol.* **33**, 732–738
- Farquhar, M. G. (1971) *Mem. Soc. Endocrinol.* **19**, 79–124
- Farquhar, M. G., Reid, J. J. & Daniell, L. W. (1978) *Endocrinology* **102**, 296–311
- Frantz, W. L. & Sonnenschein, C. (1974) *J. Cell Biol.* **63**, 103a
- Gay, V. L., Midgley, A. R. & Niswender, G. D. (1970) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **29**, 1880–1887
- Hill, M. K., MacLeod, R. M. & Orcutt, P. (1976) *Endocrinology* **99**, 1612–1617
- Jones, A. E., Fisher, J. N., Lewis, U. J. & VanderLaan, W. P. (1965) *Endocrinology* **76**, 578–583
- Labrie, F., Pelletier, G., Lemay, A., Borgeat, P., Barden, N., Dupont, A., Savary, M., Côté, J. & Boucher, R. (1973) *Karolinska Symp. 6th: Research Methods in Reproductive Endocrinology*, 301–340
- MacLeod, R. M. & Abad, A. (1968) *Endocrinology* **83**, 799–806
- MacLeod, R. M. & Lehmeier, J. E. (1972) in *Lactogenic Hormones* (Wolstenholme, G. E. W. & Knight, J., eds.), pp. 53–76, Churchill-Livingstone, Edinburgh and London
- Morgan, J. F., Morton, H. J. & Parker, R. C. (1950) *Proc. Soc. Exp. Biol. Med.* **73**, 1–8
- Neill, J. D. (1970) *Endocrinology* **87**, 1192–1197
- Nicoll, C. S. & Swearingen, K. C. (1970) in *The Hypothalamus* (Martini, L., Motta, M. & Fraschini, F., eds.), pp. 449–462, Academic Press, New York
- Pasteels, J. L. (1972) in *Lactogenic Hormones* (Wolstenholme, G. E. W. & Knight, J., eds.), pp. 269–277, Churchill-Livingstone, Edinburgh and London
- Pelletier, G., Lemay, A., Béraud, G. & Labrie, F. (1972) *Endocrinology* **91**, 1355–1371
- Shenai, R. & Wallis, M. (1974) *Biochem. Soc. Trans.* **2**, 955–956
- Shenai, R. & Wallis, M. (1975) *J. Endocrinol.* **67**, 54P–55P
- Wallis, M., Davies, R. V. & Daniels, M. (1978) *Biochem. J.* **169**, 669–676
- Zanini, A. & Giannattasio, G. (1972) *J. Endocrinol.* **53**, 177–178