Permissive effects of thyroid hormones on rat anterior pituitary mitotic activity

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Abstract

The anterior pituitary is active mitotically and apoptotically under basal conditions and in response to a variety of physiological and pathophysiological stimuli. Hypothyroidism in man is associated with a modest but very occasionally dramatic increase in overall pituitary size. The mechanisms underlying this reversible phenomenon remain obscure. In the present study we have examined young adult rat anterior pituitary following surgical thyroidectomy and subsequent thyroid hormone treatment and withdrawal using an extremely accurate system for quantifying directly identified mitotic and apoptotic events. Despite the expected increase in the number and/or proportion of immunohistochemically identifiable thyrotrophs three weeks after thyroidectomy, mitotic and apoptotic activity remained unchanged, as did pituitary wet weight, in comparison with sham-operated and intact controls. In contrast, mitotic but not apoptotic activity was enhanced by treatment of thyroidectomized animals with thyroid hormones (triiodothyronine (T_3) and thyroxine (T_4) 1.8 μg and 3.6 μg /100 g body weight per day respectively), and once again declined to levels seen in intact animals within 72 h of subsequent thyroid hormone withdrawal. Thyroid hormone-induced enhancement of mitotic activity was also seen in intact rats treated with similar doses of thyroid hormones for 7 days and in

thyroidectomized rats treated for a similar period with very low dose thyroid hormone replacement at a level that had no effect on raised hypothalamic TRH- or pituitary TSH β -transcript prevalence (0.018 μg T_3 plus 0.036 μg T_4 /100 g body weight per day). Thus changes in mitotic and apoptotic activity are unlikely to be the principle mechanism for the apparent increase in thyrotrophs up to 4 weeks after thyroidectomy. In contrast, the data indicate that thyroid hormones have a permissive effect on anterior pituitary mitotic activity in thyroidectomized male rats. Thyroid hormone-induced enhancement of mitotic activity in intact rats further suggests that in euthyroid rats, ambient thyroid hormone levels are a limiting factor for anterior pituitary mitotic activity. In summary, this time course study of young, male rats has shown for the first time that thyroidectomy, thyroid hormone replacement and subsequent withdrawal has no significant effect on anterior pituitary apoptotic activity. Secondly, it has shown that the anterior pituitary mitotic response to thyroidectomy is blocked by complete thyroid hormone deprivation, but can be restored by very low level thyroid hormone replacement, and thirdly that in intact animals thyroid hormone levels significantly limit anterior pituitary mitotic activity.

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Introduction

The adult pituitary gland is mitotically and apoptotically active under basal conditions and able to respond rapidly and repeatedly to specific stimuli such as adrenalectomy and timed glucocorticoid replacement (Nolan & Levy 2001, 2003). Modest pituitary enlargement is well described in human hypothyroidism and several case reports of pituitary enlargement sufficient to present with visual impairment through compression of the optic chiasm have been published (Ghannam *et al.* 1999, Brandle & Schmid 2000, Nicholas & Russell 2000, Kocova *et al.* 2001). Irrespective of the extent of pituitary enlargement, thyroid hormone replacement in the clinic appears to result in the rapid return of pituitary size to

normal. A similar increase in pituitary size in chronic hypothyroidism (over several months) has been reported in rats although absolute changes in pituitary weight rather than pituitary weight relative to body weight tend to be small (Quintanar-Stephano & Valverde 1997).

Several parenchymal cell subpopulations are thought to be involved in the pituitary mitotic response to hypothyroidism and thyroid hormone replacement (DeFesi *et al.* 1979, Quintanar-Stephano *et al.* 1999) and fluctuations in intracellular hormone levels that potentially cross immunohistochemical detection thresholds together with changes in relative cell size conspire to confound accurate, relative quantification (Levy & Lightman 2003). Furthermore, the identification of the secretory subtype of cells unequivocally undergoing apoptosis is inherently insecure,

and the potential for the absence of thyroid hormone to non-specifically inhibit mitotic activity must be taken into account (Ozawa & Kurosumi 1993, Quintanar-Stephano & Valverde 1997). Not surprisingly, the events that lead to the increase in relative pituitary size and subsequent resolution with treatment under these circumstances are not well understood.

We have previously described in detail the trophic responsiveness of the anterior pituitary to manipulation of the hypothalamo-pituitary-adrenal axis (Nolan *et al.* 1998, 1999, Nolan & Levy 2001, 2003). In the current study, we have examined the time course of changes in the prevalence of anterior pituitary mitotic and apoptotic events in young, male Wistar rats following surgical thyroidectomy and relatively high dose thyroid hormone replacement and subsequent withdrawal. We have also examined the effects of very low dose thyroid hormone replacement in thyroidectomized rats (insufficient to affect raised thyrotrophin releasing hormone (TRH)- and thyroid-stimulating hormone beta (TSH β)-transcript levels) and the effects of thyroid hormone treatment on mitotic activity in intact rats. Identification of these relatively rare events was achieved in thin haematoxylin and eosin-stained tissue sections using well-defined morphological criteria, and quantified using a well-validated computerized aid to manual counting.

This study is the first to examine an extended time course of anterior pituitary mitotic responses to thyroidectomy, thyroid hormone replacement and subsequent withdrawal. It is also the first to simultaneously quantify the apoptotic responses of the anterior pituitary to these various stimuli. We have also explored the permissive effects of thyroid hormones in intact rats and of very low dose thyroid hormones in thyroidectomized animals.

Materials and Methods

Animals and treatments

All animal procedures were carried out in accordance with the UK Home Office animal welfare regulations. Male Wistar rats weighing between 100 and 125 g were purchased from Bantin and Kingman Universal Ltd (Hull, UK) and allowed to acclimatise for one week before being surgically thyroidectomized or sham-operated under anaesthesia with an intraperitoneal injection (1 ml/100 g body weight (BW)) of a mixture of 2:2:2 tribromoethanol (2% w/v) in 100% ethanol (8% v/v) and 2-methylbutan-2-ol (1.2% v/v) in 0.9% saline. Thyroidectomized rats were given 1% calcium chloride to drink after surgery.

At daily intervals beginning three weeks after surgery, groups of rats were given intraperitoneal injections of a mixture of 3,3',5-triiodo-L-thyronine (T₃; 1.8 µg/100 g BW per day; Sigma) and L-thyroxine (T₄; 3.6 µg/100 g BW per day; Sigma) in a final volume of 0.4 ml saline. This dose is at least fourfold higher than that believed to be

required to normalize circulating and tissue levels of thyroid hormones (Escobar-Morreale *et al.* 1996). One week after the start of treatment, further groups of thyroidectomized animals had thyroid hormones withdrawn for an interval of up to 6 days.

To examine whether the prevalence of mitotic cells is influenced by a combination of low circulating levels of thyroid hormones in the presence of increased endogenous TRH, groups of thyroidectomized rats were given low doses of T₃/T₄ at daily intervals for 7 days starting immediately after surgery. Two doses of thyroid hormones were given: either 1.8 ng T₃ plus 3.6 ng T₄/100 g BW per day (dose A: 0.1% of the treatment dose used above), or 18 ng T₃ plus 36 ng T₄/100 g BW per day (dose B: 1% of the treatment dose).

In order to follow cumulative changes in the number of recently divided anterior pituitary cells, additional groups of rats received daily intraperitoneal injections of bromodeoxyuridine (BrdU; 10 mg/ml in 0.007 M NaOH/0.9% NaCl; Roche) at a dose of 200 mg/kg BW. Injections were started on the day of thyroidectomy or sham surgery and continued for 7 days.

Groups of rats were killed by stunning and decapitation at intervals from 24 h to 4 weeks following either surgery or the start or withdrawal of thyroid hormone treatments. Animals were killed immediately after removal from their cages in a separate room adjoining that in which they had been housed. Animals subjected to surgical thyroidectomy were examined in each case to confirm complete excision. The number of animals at each time point was between 4 and 10.

Preparation of tissue sections

Immediately after decapitation, brains were frozen on dry ice and pituitary glands were carefully removed, weighed and fixed in 4% formaldehyde in phosphate-buffered saline (PBS) for 48 h, washed in two changes of fresh PBS and embedded in 1% agar before being processed for paraffin wax embedding. A series of 2-µm thick axial sections were cut from each pituitary for histological analysis and for *in situ* hybridization histochemistry to confirm the expected changes in TSH β transcript levels. Coronal brain sections (12 µm thick) through the paraventricular nucleus were taken for TRH transcript quantification.

In situ hybridization histochemistry

In situ hybridization histochemistry was carried out as described (Levy & Lightman 1988a) using ³⁵S-dATP 3'-end-labelled synthetic 48 mer oligonucleotide probes complementary to either TRH (2 × 10⁵ counts/slide) or TSH β (2 × 10⁵ counts/slide) mRNA (Shi *et al.* 1994). The resulting images were analysed densitometrically using the program NIH Image (NIMH,

Bethesda, MD, USA) and results were expressed as the mean $\pm$ standard error relative to controls ($\% \pm$ S.E.).

TSH immunohistochemistry Pituitary sections were deparaffinized in xylene and rehydrated through a graded series of alcohols followed by incubation in 0.1 M phosphate buffer. Endogenous peroxidases were inactivated in phosphate buffer containing 20% methanol, 0.2% Triton X-100 and 1.5% hydrogen peroxide for 30 min. Sections were blocked in 1% normal goat serum and incubated overnight at 4 °C with rabbit anti-rat TSH serum (1/2000, Lot# AFP1274789, kindly provided by Dr A F Parlow of the NIDDK). After washing in 3 changes of buffer, the sections were incubated for 90 min at room temperature with biotinylated anti-rabbit IgG (Vector Labs, Peterborough, Cambs, UK; 1/200 diluted in blocking serum). Following a further 3 washes in buffer, sections were incubated with biotinylated–streptavidin–horse radish peroxidase complex (1/200 in phosphate buffer; Amersham, UK) for 90 min at room temperature. Immunoreactive cells were visualized using the 3',3'-diaminobenzidine (DAB) glucose oxidase method as described (Shu *et al.* 1988). The resulting brown colour reaction was stopped in water and sections were counterstained with haematoxylin.

BrdU immunohistochemistry Pituitary sections were processed for BrdU immunohistochemistry according to a previously published protocol with minor modifications (Cameron & McKay 1999). Briefly, de-waxed and rehydrated sections were transferred to a hot antigen unmasking solution (0.01 M citric acid in water; pH 6.0) and incubated for 10 min in a microwave oven on a power setting that maintained the solution just below its boiling point. Sections were then cooled in water to room temperature before permeabilizing for 10 min in 0.001% trypsin (Cat# 210234, Lot# 14986800, Roche, UK) diluted in 0.1% CaCl_2 /20 mM Tris buffer (pH 7.5). Following 3 washes in PBS, the slides were denatured in 2 M HCl in PBS for 30 min, washed again in PBS, gently agitated for 20 min in blocking serum (3% normal horse serum, 0.5% Triton X-100 in PBS) and incubated overnight at 4 °C with monoclonal clone B44 anti-BrdU antibody (Cat# 347580, Lot# 39497; BD Biosciences, Oxford, Oxon, UK; 1/100 diluted in blocking serum). Sections were washed in 3 changes of PBS, incubated for 1 h at room temperature with biotinylated anti-mouse IgG (Cat# BA-2001, Vector Labs; 1/200 diluted in blocking serum), and washed again in fresh PBS before blocking endogenous peroxidases for 30 min with 0.6% (v/v) hydrogen peroxide in PBS. Following a further 3 washes in PBS, sections were incubated with Vectastain Elite ABC reagent (Cat# PK-7100; Vector Labs) for 30 min at room temperature, rinsed in PBS and developed for 8 min in DAB substrate according to the manufacturer's instructions (Cat# SK-4100; Vector Labs). The resulting brown

colour reaction was stopped in water and sections were counterstained with haematoxylin. The number of BrdU-labelled cells was expressed as a percentage of total cell numbers counted (BrdU-labelling index: $\% \pm$ S.E.).

Image analysis for trophic activity

Apoptotic, mitotic and BrdU-immunopositive cell counts were performed on 2 μm -thick haematoxylin and eosin (H&E)-stained rat pituitary sections at 1000 $\times$ magnification with the aid of a dedicated real-time computer system to tag and tally the co-ordinates of manually identified trophic events within each tissue section (Nolan *et al.* 1998). The computerized Highly Optimized Microscope Environment (AxioHOME, Zeiss (Brugal *et al.* 1992)) that was used, projects a virtual image of the computer screen which appears fractionally above the actual microscope image and allows different markers to be laid down over apoptotic, mitotic or normal cells by hand as the stage is moved. The system retains a cumulative score of the numbers of each cell type counted, together with the co-ordinates of each individually marked cell irrespective of the position of the microscope stage or changes in power of the objective lens. Extremely accurate quantification of the various cell types is thus possible for each section studied, as areas can be circumscribed at low power (eliminating selection bias), and counted at high power without danger of double scoring.

The histological markers that were used to identify apoptotic cells were clusters of two or more apoptotic bodies consisting of extremely dense round or oval structures varying in diameter from approximately 0.7 to 4 μm and surrounded by normal cells. Earlier stages of apoptosis cannot be visualised using H&E staining and light microscopy. For each animal, three random areas of approximately 47 000 μm^2 were scored for the presence of mitotic and apoptotic figures. The error in quantifying the number of normal cells surrounding these events was $\leq 2\%$.

TSH immunopositive cell counts were performed at 400 $\times$ magnification. For each animal, four areas of approximately 75 000 μm^2 , each containing an average of 800 cells, were scored as positive or negative for the presence of immunoreactive TSH. No corrections were made for any changes in thyrotroph cell size that occur following thyroidectomy (Floderus 1944).

All slides were coded and counted by one observer (L A N) blinded to the treatments. Results were expressed as a percentage of the total cell numbers counted for each animal.

Statistics

Data were expressed as means $\pm$ S.E. GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used to perform statistical calculations. Differences between

groups were evaluated using one-way ANOVA followed by Tukey–Kramer multiple comparison post-tests. $P < 0.05$ was considered statistically significant.

Results

Thyroidectomy and thyroid hormone replacement efficacy

Surgical thyroidectomy was associated with a consistent sixfold increase in both paraventricular TRH transcripts and anterior pituitary TSH β transcripts ($P < 0.001$, $n = 4-6$; Fig. 1a,b). No thyroid remnants were found in any of the thyroidectomized animals on post-mortem examination. Three days after the start of high dose thyroid hormone treatment, both TRH and TSH β transcript levels had fallen to below control values and remained suppressed until 4 days after treatment was withdrawn (Fig. 1a,b), indicating a relatively short biological half life of circulating thyroid hormones (including T₄) in these animals.

Low dose (18 ng T₃ plus 36 ng T₄/100 g BW per day) and very low dose thyroid hormone replacement (1.8 ng T₃ plus 3.6 ng T₄/100 g BW per day) – equivalent to between 1.2% and 12% of the dose of T₃ and 0.4 to 4% of the dose of T₄ required to normalize circulating TSH and T₄ and T₃ levels in plasma and tissues (Escobar-Morreale *et al.* 1995, 1996) was insufficient to diminish raised TRH and TSH β transcript levels (Fig. 2a,b).

Pituitary weight changes

Pituitary wet weight increased by 33% between days 3–4 and day 21 of the study (from an average of 5.54 ± 0.09 mg to 7.37 ± 0.18 mg) in keeping with overall somatic growth. There was no significant difference in gain in pituitary weight between thyroidectomized and sham operated animals over this time period (7.29 ± 0.23 mg vs 7.43 ± 0.26 mg at 21 days).

TSH immunohistochemistry

Under basal conditions, using a standard formaldehyde fixation procedure, $4.31 \pm 0.15\%$ of the total parenchymal cells in the anterior pituitary were immunohistochemically identifiable thyrotrophs. Three weeks after surgical thyroidectomy, the number of thyrotrophs appeared to have increased to $6.39 \pm 0.47\%$ ($P < 0.01$; $n = 6$). One week after supraphysiological thyroid hormone replacement, the number of thyrotrophs had decreased to $5.48 \pm 0.32\%$. However, cell counts were not corrected for the increase in thyrotroph cell size that occurs after surgical thyroidectomy, and will therefore be an overestimate of changes in thyrotroph cell numbers (Floderus 1944).

Changes in anterior pituitary mitotic and apoptotic activity

In control (untreated and intact) animals, the prevalence of mitotic figures and apoptotic bodies in the anterior

pituitary was $0.086 \pm 0.013\%$ and $0.036 \pm 0.008\%$ respectively ($n = 10$; Fig. 3a,b), equivalent to an average rate of anterior pituitary cell entry into mitosis of approximately once every 60–70 days (Nolan *et al.* 1999). There were no significant differences in the prevalence of these trophic markers following surgical thyroidectomy at any time-point up to 28 days after surgery.

Following treatment with a high dose of thyroid hormones beginning 21 days after thyroidectomy, there was a significant increase in mitotic activity that peaked 5 days after the start of treatment at $0.231 \pm 0.064\%$ (Fig. 3a). There was no concurrent, significant increase in apoptotic activity over the same time period. Following withdrawal of thyroid hormone treatment, the prevalence of both mitotic figures and apoptotic bodies dipped below baseline levels although the values did not reach statistical significance compared with sham-operated controls.

To determine whether independent of their specific effects on the hypothalamic–pituitary–thyroid axis, thyroid hormones may be required for anterior pituitary cell proliferation to occur, low dose and very low dose T₄ and T₃ at 1% and 0.1% of the treatment dose (less than 5% of estimated physiological replacement dose) were given for 7 days after surgical thyroidectomy. This resulted in a small, dose-related incremental increase in the mitotic index measured 7 days later (Fig. 4), consistent with a permissive effect of thyroid hormones on mitotic activity in the presence of raised TRH levels.

As the total number of mitotic figures observed at any one time point remained extremely low at a fraction of one percent, it was not possible to, and neither did we expect to be able to, correlate mitotic activity with specific cellular subpopulations with sufficient precision to allow any conclusions to be drawn about the cell type or types involved.

Cumulative mitotic activity measured with BrdU incorporation

The absence of any overall change in mitotic activity during the seven days after thyroidectomy was confirmed by measuring the cumulative BrdU-labelling index (Fig. 5). Although no significant difference in the number of BrdU-labelled cells was found between thyroidectomized and intact control animals, intact rats treated with a high dose of thyroid hormones over a similar time period showed a significant increase in anterior pituitary cell BrdU-labelling index. The absolute percentages of BrdU-labelled cells measured may be affected by many of the stages of the immunohistochemistry procedure, in particular the characteristics of the primary antibody used. The assay was therefore carried out simultaneously on all sections so that valid comparisons could be drawn between the three experimental groups.

Figure 1 The effects of surgical thyroidectomy, supraphysiological thyroid hormone treatment ($1.8 \mu\text{g T}_3$ and $3.6 \mu\text{g T}_4$ per 100 g BW per day) and thyroid hormone withdrawal on the prevalence of paraventricular TRH transcripts (a) and anterior pituitary TSH β transcripts (b). Mean deviation from controls (Con) is shown as $\% \pm \text{S.E.}$, $n=4-6$. $**P<0.01$, $***P<0.001$ compared with controls. Representative autoradiographs of the paraventricular nucleus (a) and pituitary (b) from different groups are shown. The scale bars are 1 mm.

Discussion

The rate of change in the size of a specific cell population is determined not only by the rate of cell proliferation and differentiation into the compartment of interest, but also

by the rate of apoptosis. Real changes in the relative size of pituitary subpopulations are also potentially influenced by transdifferentiation, a direct change from one mature phenotype to another through qualitative changes in hormone production by a dedicated, unihormonal cell.

Figure 2 Lack of effect of continual, low dose (subphysiological) thyroid hormone replacement on TRH and TSHβ transcriptional activity in thyroidectomized (Tx) rats. Low dose and very low dose thyroid hormone replacement (either 1.8 ng T₃ plus 3.6 ng T₄, or 18 ng T₃ plus 36 ng T₄) was given daily for the week after surgical thyroidectomy. The prevalence of paraventricular TRH transcripts (a) and anterior pituitary TSHβ transcripts (b) was measured. Mean deviation from controls is shown as % ± S.E., $n=5-7$. ** $P<0.01$, *** $P<0.001$ compared with controls.

Indeed, thyrosomatotrophs, regarded by some as an intermediate cell in the transdifferentiation between somatotrophs and thyrotrophs, have been demonstrated in both human (Vidal *et al.* 2000) and rat (Horvath *et al.* 1990) pituitary in response to protracted hypothyroidism.

In the present study we have shown that surgical thyroidectomy, unlike surgical bilateral adrenalectomy (Nolan *et al.* 1998, Nolan & Levy 2001), does not induce an overt increase in anterior pituitary mitotic activity that can be measured morphologically. This was despite a sixfold increase in both pituitary TSHβ and paraventricular TRH transcript prevalence, and a presumed concomitant increase in pituitary TRH peptide exposure.

These data were further corroborated by the lack of an increase in the cumulative BrdU-labelling index one week after thyroidectomy when compared with the cumulative BrdU-labelling index found in intact controls. In addition, the rate of apoptosis remained at a level indistinguishable from the baseline rate throughout the 3-week period following total thyroidectomy.

The proportion of anterior pituitary immunohistochemically identifiable thyrotrophs was markedly increased when measured three weeks after thyroidectomy. However, larger structures are relatively over-represented in tissue section as they are more likely to appear and to be counted in multiple sections – a factor exemplified in the extreme by considering over-representation in section of a single tennis ball in a box of 1000 table tennis balls. Thus the real increase in prevalence of thyrotrophs per unit volume of the anterior pituitary is likely to be considerably more modest than the absolute percentages measured in this study by direct counting.

Furthermore, although not directly addressed in this study, changes in the number of thyrotrophs that may have resulted from transdifferentiation would be immunohistochemically indistinguishable from quantitative switching in hormone production by bihormonal or pleurihormonal cells. Given the high prevalence of transcriptionally pleurihormonal tumours that clinically lead to hypersecretion of only one hormone (Levy & Lightman 1988*b,c*), and the apparent multi-responsiveness of individual anterior pituitary cells to hypothalamic-releasing hormones (Villalobos *et al.* 1997), it is possible that many cells classified as dedicated single hormone secreting are in fact bihormonal or pleurihormonal but that only one hormone is present at an immunochemically detectable level.

In male rats that have been thyroidectomized 5 months previously, thyroid hormone replacement and exogenous TRH act synergistically to increase the proliferative response in somatotrophs (Quintanar-Stephano & Valverde 1997). It has also been suggested that T₄ is required for normal somatotroph proliferation to occur (Quintanar-Stephano *et al.* 1999). Our results are in agreement with these data in as much as we were able to demonstrate a small but significant increase in mitotic activity beginning two days after the start of supra-physiological thyroid hormone treatment in hypothyroid rats. Once again, the rate of apoptosis remained unchanged throughout the week-long treatment period. Following withdrawal of thyroid hormones, the mitotic index fell rapidly to a level below that found in intact controls. Thus thyroid hormones appear to exert a general permissive effect on mitosis in the thyroidectomized rat anterior pituitary in the presence of high levels of TRH. The addition of very low dose thyroid hormone replacement in thyroidectomized animals (approximately 12% of the T₃ and 4% of the T₄ doses required to

Figure 3 The effects of alterations in thyroid status on anterior pituitary mitotic and apoptotic activity. Changes in the prevalence of mitotic cells (a) and apoptotic cells (b) are shown as a percentage of total anterior pituitary parenchymal cells following surgical thyroidectomy or sham surgery (at time zero), thyroid hormone replacement from day 21 to day 28 and thyroid hormone withdrawal from day 28 to day 35. Values shown are means $\pm$ S.E., $n \geq 6$; ** $P < 0.01$ compared with controls at time 0.

maintain circulating and tissue thyroid hormone levels (Escobar-Morreale *et al.* 1996)) induced a small but significant increase in the prevalence of mitotic figures after one week. Raised levels of TRH are not required to permit thyroid hormone-mediated mitotic effects in the anterior pituitary, however, as supraphysiological levels of thyroid hormones in intact rats also significantly enhanced the anterior pituitary BrdU-labelling index over a period of one week. The data obtained from intact rats also indicate that under euthyroid conditions in young male rats, ambient thyroid hormone levels to some extent constrain pituitary mitotic activity, at least in the short term.

At any one time point the maximum percentage of cells identified as being in mitosis is less than a quarter of one percent, a level that prevents accurate quantification of the cell types involved in proliferation. We were therefore unable to exclude the possibility that thyroidectomy

might result in a switch in mitotic activity towards thyrotrophs, with a concurrent reduction in mitotic activity in other cellular subpopulations, such as lactotrophs and somatotrophs (Astier *et al.* 1980, Stahl *et al.* 1999) leaving overall mitotic levels unchanged. Unlike the case in humans, particularly females, where hypothyroidism is associated with hyperprolactinaemia and some degree of lactotroph hypertrophy (Pioro *et al.* 1988, Molitch 1992, Ghannam *et al.* 1999, Brandle & Schmid 2000, Kocova *et al.* 2001, Raber *et al.* 2003), lactotrophs tend to atrophy post thyroidectomy in rats (Ozawa & Kurosuni 1993, Kimura & Furudate 1996). In our study, anterior pituitary prolactin transcripts were reduced during the onset and maintenance of hypothyroidism and returned to control levels or above after the start of thyroid hormone replacement (data not shown).

In summary, we have demonstrated that there is no overall increase in mitotic or apoptotic prevalence in the

Figure 4 The stimulatory effects of continual, low dose (subphysiological) thyroid hormone replacement on the directly measured prevalence of mitotic cells in thyroidectomized (Tx) rats. Low dose and very low dose thyroid hormone replacement (either 1.8 ng T₃ plus 3.6 ng T₄, or 18 ng T₃ plus 36 ng T₄) was given daily for a week following surgical thyroidectomy. Values shown are means $\pm$ S.E., $n > 5 \leq 10$; * $P < 0.05$.

male rat anterior pituitary during the immediate period after surgical thyroidectomy despite an apparent increase in the number of thyrotrophs. Thyroid hormones appear to be permissive for pituitary mitotic responses but increased TRH alone has no effect. Finally, our results suggest that under basal conditions, ambient thyroid hormone levels restrain anterior pituitary mitotic activity.

Figure 5 The cumulative effects of supraphysiological thyroid hormone treatment on anterior pituitary BrdU-labelling index in intact animals after 7 days. Intact rats were given 1.8 µg T₃, 3.6 µg T₄ and 20 mg BrdU per 100 g BW per day, daily for 7 days, prior to analysis of BrdU-labelling index. Means $\pm$ S.E. are shown, $n = 4-5$; * $P < 0.05$ compared with controls. Tx, thyroidectomized.

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