Physiological implications of pituitary trophic activity

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Abstract

A complete inventory of pituitary trophic responses depends on precise estimates of mitotic activity and apoptotic events, and accurate characterization and quantification of pituitary cell subtypes irrespective of previous and current physiological demand. For a discrete structure that has been so extensively studied, it is disappointing but perhaps not surprising that none of these measures is available and therefore that the relative contributions to changes in the proportions of pituitary cellular subpopulations of trophic activity, differentiation of pluripotent cells and variations in the secretory profiles of apparently committed cells remain almost impossible to determine. To fully appreciate the extent of this dilemma, it should be remembered that conservative estimates of the proportion of corticotrophs in the rat anterior pituitary under basal conditions vary over twofold and that it is still not clear whether the apparent threefold increase in defined corticotroph cells in the rat pituitary. Although these tend to be stellate rather than compact, as corticotrophs constitute one of the smaller and more discrete cellular subpopulations, they should be amongst the most straightforward to quantify. Even so, estimates of corticotroph numbers in normal young rats under unstimulated conditions vary from less than 3% (Dada et al. 1984, Taniguchi et al. 1995) to more than 20% (Castro et al. 1995) with a greater than twofold difference in approximations even if these extremes are excluded (4·5 ± 0·3% (Gertz et al. 1987), 4·7 ± 0·28% (Nolan et al. 1998), 6·2 ± 0·3% (Asa et al. 1992a), 7–10% (Childs 1987) and 9·7 ± 1·0% (Westlund et al. 1985)). Whether, in addition to differences in age, sex, strain and animal handling, this variability in part reflects disparities in absolute corticotroph numbers or alterations in total cell numbers in the pituitary is unanswered and very difficult to address.

Accurate counting is even more demanding with the prevalent parenchymal subtypes such as somatotrophs and mammotrophs owing to the proximity of similar cells and larger scale topographical organization. Accordingly, in

Introduction

In practice, the accuracy of immunocytochemical quantification is limited by the use of secreted peptides as markers under circumstances known to produce dramatic changes in hormone content and by subtle, yet potentially highly significant, technical constraints. Quantitative immunocytochemistry is hampered by cell clumping, which frustrates accurate differentiation of individual cells and variations in cellular shape and size that limit the validity of extrapolating cell counts derived from transection planes into tissue volume. The potential for transdifferentiation (Horvath et al. 1990, Kineman et al. 1992, Vidal et al. 2000, 2001) or at least marked changes in the relative proportions of peptide markers in what seem increasingly likely to be pleuripotent cells further undermines the interpretation of immunocytochemical counting indices even if the methodology and antibodies and other reagents used could be completely standardized. The resulting variability in estimates of specific cell types is exemplified by reports of the prevalence of immunocytochemically

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man, reports of basal mammotroph prevalence in nulliparous females and adult males vary from less than 9% to over 30% (Asa et al. 1982) – a range within which important changes in cell population sizes in response to physiological stimuli could clearly be lost. Furthermore, mitotic markers such as the proliferation-associated nuclear antigen Ki-67 and S phase-incorporated bromodeoxyuridine (Gratzner 1982) both rely on immunocytochemical detection, and whilst actively cycling cells can be recognized directly in tissue sections during metaphase or identified autoradiographically after S phase exposure to tritiated thymidine, absolute quantification of trophic activity, even if no attempt is made to specifically identify the secretory subtype of the cells involved, is also a formidable task.

Flow cytometry has been used to study pituitary cell cycle kinetics (Toni & Vitale 2000) and is capable of yielding relatively pure cellular populations from dispersed pituitary cells after suitable stimulation (Wynick et al. 1990a,b). However, the technique is insensitive and it cannot realistically be used to quantify different cell types as it suffers many of the technical constraints and much of the same variability as immunocytochemistry.

Timed colchicine-induced mitotic arrest to extract mitotic rate information from fixed tissue sections (stathmokinetic analysis) can be used to provide a surrogate estimate of the relationship between apoptotic event prevalence and apoptotic rate (Kolopp et al. 1992, Nolan et al. 1998, 1999) but the poorly specified duration of cell death labelling using terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (Mangili et al. 1999) or protein markers (Kulig et al. 1999) prevents dynamic data about these processes being extrapolated from prevalence measurements. The findings that most mitotic activity occurs in cells that are not immunopositive for any of the pituitary hormones (Dada et al. 1984, Carbajo-Perez & Watanabe 1990), that the mitotic response of the pituitary to adrenalectomy is largely confined to cells that are not immunocytochemically identifiable as corticotrophs (Taniguchi et al. 1995) and that the fleeting, but quantitatively impressive, apoptotic response of adrenalectomized rats to glucocorticoids must, numerically, involve non-corticotroph cells (Nolan et al. 1998) adds further to the complexity. In short, the cellular subtypes in the pituitary that show trophic activity cannot be directly recognized with any facility or their identities surmised from the prevailing physiological circumstances by conjecture alone. The following brief overview of published data regarding dynamic changes in pituitary cell populations (the paucity of which reflects these difficulties) should therefore be considered with these limitations in mind.

The effects of age on pituitary trophic activity

Organogenesis is a primary but transient trophic demand in early life. From around 5·5 ± 0·1 mg (mean ± S.E.) in weight at 6 weeks, in our animal facility and fed standard laboratory diet (which is designed to modestly restrict growth), the male Wistar rat pituitary doubles in weight to 11·4 ± 0·2 mg at 6 months and increases by a further 10% over the next 18 months, to 12·6 ± 1·3 mg by 24 months (Fig. 1). A combination of enzymatic cellular dispersal, direct cell counting, DNA determinations and electron microscopic differential cell counting has provided an estimate of total anterior pituitary cell numbers in rats at this age (Surks & DeFesi 1977). As the proportion of the pituitary taken up by vascular and connective tissue structures and the overall size and packing of parenchymal cells vary little over this time, this represents an increase in cell numbers from around 1·4 × 10⁶ at 6 weeks to 3·3 × 10⁶ at 2 years. Using timed colchicine-induced mitotic arrest with accurate quantification of the prevalence of mitotic figures, it is apparent that between 6 weeks and 16 months of age, and perhaps throughout life, pituitary mitotic activity exceeds that required for organ growth. On average, just under 60% of the directly measured cell production contributes to overall pituitary growth between the ages of 6 weeks and 13 weeks. Between 13 weeks (when turnover rate averages 1·58% per day) and 6 months, 14% of nascent cells contribute to increased pituitary weight. Between 6 months and 1 year, less than 4·6% of cell production contributes to pituitary growth, and between 16 months and 2 years, less than 3% of cell production contributes to growth (LA Nolan & A Levy, unpublished findings). Thus, while the absolute rate of cell turnover decreases markedly with time, an increasing proportion of cellular turnover is not related to organogenesis per se. Studies in beagle dogs concur with a general reduction in pituitary cell turnover with age (Kolopp et al. 1992). Whether cell turnover in the rat pituitary declines continuously from the time of its first

Figure 1 Age-related changes in the intact male Wistar rat pituitary. Mean pituitary wet weight ± S.E. is shown.

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appearance in foetal life (Friend 1979) or accelerates in early post-natal life to a peak at about 4 weeks before subsequently declining (Shirasawa & Yoshimura 1982, McNicol & Carbajo-Perez 1999) is not yet entirely clear, although the latter evidence is perhaps more secure (denoted by the discontinuous line segment in Fig. 2 (Nolan et al. 1999)). It has also been suggested that different cell subpopulations have different rates of basal mitotic activity at different ages with, for example, a steadily decreasing mitotic rate for corticotrophs, apart from a peak just before 3 weeks of age and a progressive increase in somatotroph and mammotroph mitotic activity during the first 10 weeks of life (Shirasawa & Yoshimura 1982).

In addition to changes in rate of cell division, in female Wistar rats at least, there appears to be an age-related change in the proportion of cell types with mammotrophs increasing and pure somatotrophs decreasing with senescence (Shinkai et al. 1995). At the same time, the number of mammomamomatrophs increases more than tenfold in rats older than 21 months compared with rats less than 13 months old (Shinkai et al. 1995). The precise extent to which this represents a true change in cell proportions or less polarized hormone expression in chronically pleuripotent, terminally differentiated cells remains a conundrum. In man, despite a clear age-related decline in growth hormone (GH) secretion, there does not appear to be a significant decline in somatotroph cell mass (Thorner et al. 1997).

Circadian rhythmicity of trophic activity

Several studies have suggested that there is a circadian rhythm in mitotic activity in the rat pituitary. Whether peak activity occurs during the night (Carbajo-Perez et al. 1991, Oishi et al. 1993) or during the day, or as one peak of mitotic activity (McNicol & Carbajo-Perez 1999) or two (Nouet & Kujas 1975) is unclear. Differences in age, sex and breed of rats aside, the very low prevalence of mitotic figures and the unpredictable effects of stress in different holding facilities is likely to make this issue hard to resolve. The observation is of considerable interest, nevertheless, as the presence of circadian changes implies that, in addition to intrinsically driven mitotic and apoptotic activity, the pituitary is closely coupled to acute hypothalamic trophic influences.

Pregnancy and the gonadal axis

The volume of the adult human pituitary appears to be slightly greater in females than males in vivo as estimated using magnetic resonance imaging reconstruction (Denk et al. 1999) and also in autopsy series (Bergland et al. 1968), particularly following multiple pregnancies (Asa et al. 1982). During pregnancy itself, the human pituitary enlarges by about 20–36% and peaks in size several days post-partum (Gonzalez et al. 1988, Elster et al. 1991, Dinc et al. 1998). Whether these changes indicate trophic activity or changes in vascularity and/or cell size is unknown. In 10-week-old rats it has been found that anterior pituitary mitotic activity in females is twice as high as that found in males overall, with a peak of mitotic activity occurring in oestrus (Oishi et al. 1993). The distribution of mitotic activity amongst the different pituitary cell types is similar between the sexes, with mammotrophs accounting for the highest proportion of mitotic activity overall (Oishi et al. 1993). In beagle dogs, no sexual dimorphism in basal trophic activity in the pituitary gland has been found (Kolopp et al. 1992) and, predictably, no human data are available.

Stathmokinetic analysis in combination with luteinizing hormone immunochemistry has been used to demonstrate a tenfold increase in proliferating gonadotrophs 1–2 weeks after orchidectomy, resulting in a temporary increase in gonadotrophs in rats (Inoue et al. 1985). There is no evidence, however, that under normal circumstances continuous exposure to hypothalamic gonadotrophin-releasing hormone has any trophic action on gonadotrophs in vivo or in organ culture (Khar et al. 1978, Pawlikowski et al. 1978a), although an indirect gonadotroph–dependent trophic effect on lactotrophs and corticotrophs has been suggested (Denef et al. 1990, Tilemans et al. 1992).

Oestradiol significantly increases stimulated thymidine uptake by rat anterior pituitary cells in vivo (Pawlikowski et al. 1997), and case reports have associated the apparent progression of a human microprolactinoma to a macroprolactinoma (Garcia & Kapcala 1995) and the development of invasive characteristics of a macroprolactinoma (Bevan et al. 1989b) with prolonged oestrogen treatment. It is still

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not clear whether the marked increase in pituitary prolactin-secreting cells during pregnancy from 15% antenatally to 50% at term (Haggi et al. 1986) and restoration to nulliparous levels within 7–21 days (if suckling does not take place) is mediated in part by mitosis and apoptosis, or whether transdifferentiation of somatotrophs into bihormonal mammosomatotrophs (Vidal et al. 2001) or complete transformation into lactotrophs via the mammosomatotroph phenotype is entirely responsible for the changes seen (Stefaneanu et al. 1992).

The thyroid axis and trophic activity

Prolonged hypothyroidism in some patients produces a dramatic but reversible increase in pituitary size, sometimes with few other systemic symptoms of hypothyroidism (Bilaniuk et al. 1985, Abram et al. 1992, Alkhani et al. 1999, Brandle & Schmid 2000, Vidal et al. 2000). In the thyroidectomized rat model, there is a fairly consistent 25% increase in total cell numbers, with a fourfold increase in the number of thyrotrophs and concomitant reduction in the number of immunocytochemically defined somatotrophs by almost two-thirds compared with intact animals (Surks & DeFesi 1977).

Thyrotrophin-releasing hormone (TRH)-induced pituitary cell proliferation has been demonstrated in vivo (Kunert-Radek & Pawlikowski 1975, Pawlikowski & Slowinska-Klencka 1994) and in organ (Pawlikowski et al. 1975) and cell culture in vitro (Pawlikowski et al. 1975). TRH-induced tritiated thymidine incorporation in vitro is inhibited by thyroxine (Pawlikowski et al. 1975) and blocked completely by somatostatin (Pawlikowski et al. 1978a). Thyroxine itself has no effect on adenohypophyseal cell proliferation in vitro (Pawlikowski et al. 1975) but in thyroidectomized and chronically hypothyroid rats, in which additional exogenous TRH alone is ineffective in inducing a proliferative response, thyroxine replacement produces a dose-dependent increase in mitotic activity in somatotrophs and (in some respects paradoxically) in thyrotrophs, and restores the ability of TRH to induce mitotic activity at least in somatotrophs (Quintanar-Stephan & Valverde 1997).

It is still not entirely clear whether there is a direct effect of TRH on tritiated thymidine incorporation by thyrotrophs as such a response has not been detected by double labelling of monolayer anterior pituitary cell cultures exposed to TRH (Komolov et al. 1978). Thus it is conceivable that the apparent fourfold increase in the number of thyrotrophs in thyroidectomized rats (Surks & DeFesi 1977) is mediated, in part, by transdifferentiation from the somatotroph and thyrotrhop lineages, as well as appearing to result artefactually from a relative reduction in the somatotroph population. As long-term incubation with TRH abolishes the growth-inhibitory effects of dopamine on the GH4C1 prolactin- and GH-secreting rat pituitary tumour-derived cell line transfected with the short form of the D2 dopamine receptor, part of the growth-modulating effects of TRH may be mediated through indirect inhibitory effects on D2 dopamine receptor function (Senogles 1994). It is unclear whether data derived from a cell line can usefully inform the situation in vivo, but intuitively such an effect on D2 dopamine receptor function may be, in part, responsible for the stimulatory effect of TRH on proliferation in pituitary intermediate lobe cells (Pawelczyk et al. 1996).

Dopamine effects on trophic activity

Dopamine receptor antagonists such as pimozide and sulpiride enhance mitotic activity and the dopamine receptor agonist bromocriptine inhibits mitotic activity in the anterior pituitary of normal and oestrogen-treated male rats (Stepien et al. 1978, Perez et al. 1986). Increased apoptotic activity in the anterior pituitary of male Fischer rats during the first 44 h after withdrawal of chronic oestrogen treatment is further enhanced by bromocriptine treatment (Drewett et al. 1993). A similar antiproliferative effect of dopamine agonists on the anterior pituitary is seen in organ culture (Pawlikowski et al. 1978b) and in vivo under basal conditions (Lloyd et al. 1975). Intraventricular injection of the neurotoxin 6-hydroxydopamine, which depletes striatal dopamine, results in a modest increase in pituitary mitotic activity, principally in the acidophilic and chromophobe compartments, when measured 4 and 12 days (but not 2 days) after the injection (Lewinski et al. 1984). The authors speculated that the absence of effect at 2 days was related to a relative increase in hypothalamic–hypophysal portal dopamine resulting from early dopaminergic nerve damage. Whether the stimulatory effects of fibroblast growth factor on mitotic index in anterior pituitary explants and the inhibitory effects of bromocriptine on fibroblast growth factor-induced mitosis affect the same cells remains open to speculation (Pawlikowski & Stepien 1979).

The effects of GH- and corticotrophin-releasing hormones, glucocorticoids and vasopressin on pituitary trophic activity

GH-releasing hormone (GHRH) has trophic activity in the human (Guillemin et al. 1982, Thorner et al. 1982, Billestrup et al. 1986, Sano et al. 1988) and mouse pituitary (Mayo et al. 1988, Asa et al. 1990, 1992b, Struthers et al. 1991, Lloyd et al. 1992). Prolonged exposure to relatively high levels of GHRH may be limited to hyperplasia (Ezzat et al. 1994) or induce true somatotroph adenoma formation (Asa et al. 1984, Bevan et al. 1989a). Ultimately, however, the distinction might not be relevant, as hyperplasia and adenoma formation may not be distinct processes (Derwahl & Studer 2001).
Corticotrophin-releasing hormone (CRH) has unequivocal trophic potential in the pituitary (Carey et al. 1984, Childs et al. 1995) and chronic administration has been associated with increased corticotroph numbers (Gertz et al. 1987, Asa et al. 1992a), corticotroph nodular hyperplasia (in untreated Addison’s disease) (Scheithauer et al. 1983) and, in a single case report of an intrasellar CRH-producing gangliocytoma, with corticotroph adenoma formation (Saeger et al. 1994). On its own at low concentrations, arginine vasopressin (AVP) is a more potent inducer of bromodeoxyuridine uptake into rat anterior pituitary cells than CRH, and low levels of CRH enhance low-dose AVP-induced bromodeoxyuridine incorporation (McNicol et al. 1990).

The effects of adrenalectomy and glucocorticoid replacement on trophic activity in the rat anterior pituitary gland have been investigated for over 20 years (Stepien et al. 1981). Using simultaneous immunohistochemistry for adrenocorticotropic hormone and bromocriptine, the latter given as a bolus 3 h before the animals were killed, overall mitotic activity has been shown to increase during the week after adrenalectomy and decrease again by 2 weeks leading to a 1.5-fold increase in the number of immunocytochemically identifiable corticotrophs (Taniguchi et al. 1995, Nolan et al. 1998). Closely associated with the transient increase in mitotic activity, maximal between 3 and 7 days post-adrenalectomy (Fig. 3a), is a progressive increase in the population of

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Figure 3 The effects of high-dose glucocorticoids on anterior pituitary mitotic and apoptotic activity in male Wistar rats. (a) The effects of adrenalectomy and subsequent high-dose glucocorticoid replacement on mitotic and apoptotic activity. (b) The effect of high-dose glucocorticoid treatment and glucocorticoid withdrawal in intact controls. Redrawn from Nolan & Levy (2001). Glucocorticoid-induced apoptotic bursts are highlighted in yellow.

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Figure 4 Schematic diagram of changes in pituitary mitotic activity and apoptotic responsiveness to glucocorticoids following bilateral adrenalectomy. (a) Changes in the prevalence of mitotic activity over time, with a peak from 3 to 7 days returning spontaneously to levels seen in intact animals by 2 weeks. (b) Time-related changes in the size of the populations of cells that undergo glucocorticoid-inducible apoptosis after adrenalectomy. A progressive increase during the first week reaches a plateau between 1 and 2 weeks after adrenalectomy before declining to levels seen in untreated intact animals at 4 weeks.
cell death that undergo apoptosis in response to dexamethasone (compare the heights of the early (≤72 h) dexamethasone-induced apoptotic responses in Fig. 3a and b). This dexamethasone-sensitive population reaches maximum size after the mitotic peak and plateaus at three times sham-operated controls between 1 and 2 weeks post-adrenalectomy before spontaneously returning to baseline levels by 4 weeks post-surgery (Fig. 4). This suggests that mitotic activity is not only temporally constrained, in as much as it changes for a limited and reproducible period after adrenalectomy despite continued absence of corticosterone and continued elevation of CRH transcription, but also that mitotic and apoptotic activity in the pituitary are closely linked to each other, at least under these circumstances, with apoptotic sensitivity seemingly confined to cells that have entered the cell cycle within the previous 7 to 14 days (Fig. 5) (Nolan & Levy 2001).

**Other potential trophic modulators**

Prostaglandins have complex effects on many aspects of pituitary and hypothalamic function, including modulation of interleukin 1-stimulated CRH release (Watanobe et al. 1995) and stimulation of plasma prolactin levels when injected intracerebroventricularly (Costa et al. 1979, Tojo et al. 1986). Indomethacin, an inhibitor of prostaglandin synthesis, inhibits oestrogen-induced DNA synthesis in the rat anterior pituitary gland (Pawlikowski et al. 1981), yet intracerebroventricular injection of prostaglandin E2 itself seems to result in a significant decrease of mitotic activity in anterior pituitary chromophobic cells (Sewerynek et al. 1986).

The trophic effects of oestradiol in vivo on pituitary cells was found to be enhanced by concurrent treatment with the peripheral benzodiazepine receptor ligand 4′-chlorodiazepam (Ro 5-4864) but not by the central-type benzodiazepine receptor antagonist Ro 15-1788 (Stepien et al. 1986). A single injection of diazepam reduced anterior pituitary cell mitotic activity in 11-day-old female rat pups (Pawlikowski et al. 1987) and was found to suppress tritiated thymidine uptake in vitro (Kunert-Radek et al. 1994). These findings suggest that benzodiazepine receptors may be involved in the control of pituitary cell proliferation. If so, the cell type involved and the physiological consequences of
benzodiazepine use at the level of the pituitary remain unknown.

Stimulation of tritiated thymidine incorporation into rat pituitary tumour cells has been demonstrated for gastrin fragments (Pawlowski et al., 1992), and tritiated thymidine incorporation into normal rat anterior pituitary cells stimulated by angiotensin II. Saralasin, an angiotensin II antagonist, has been found to suppress angiotensin II-induced mammosomatotroph proliferation in some (Shinkai & Ooka 1995) but not all studies (Pawlowski & Kunert-Radek 1997).

In summary, until stable, non-secreted, simultaneously identifiable and easily distinguishable markers for each different anterior pituitary cell type become available, distinguishing between transdifferentiation, quantitative fluxes in relative hormone gene expression in pleuripotent cells, maturation of previously uncommitted cells and physiological and potentially pathophysiological trophic responses within the pituitary will remain a technical impasse (Levy 2000, 2001, Derwahl & Studer 2001). While it does, the long-term consequences of short-term fluctuation in pituitary trophic activity will remain obscure and, for example, the utility of using array technology to identify quantitative differences in gene expression between the normal pituitary and pituitary adenomas will continue to be hampered by difficulty in correcting for cell-specific variations amplified by polarization of cell populations in clonal expansions. This fundamental goal is nevertheless important to pursue as the effects on pituitary responsiveness of age, sex and temporarily different patterns of pharmacological interventions such as intermittent glucocorticoid exposure, as well as potential insights into the pathogenesis of pituitary adenomas, may depend on it.

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