Cell size and cell division of the anterior pituitary: Time course in the growing rat[1]

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Summary. Pituitary cells increase their numbers more than 3-fold during the 1st 10 days of life while maintaining the same cell size ratios. In the 25-day-old animal, the rate of cell division slows and there is a slight increase in the number of larger cells. An increase in adult weight is attributed to hyperplasia and a shift to a population of larger cells.

Organ growth is a complex process which may involve cellular hypertrophy and hyperplasia. The interaction of these 2 phenomena has been studied in relatively few organs, e.g. heart, liver and salivary glands[2-4]. Little information is available on endocrine gland growth. The anterior pituitary is accessible, self-contained and, therefore, an ideal gland to investigate. In addition, the pattern of mitosis and DNA synthesis in the anterior pituitary of growing rats has been established[5-6]. This information could be correlated with data collected in this study. The purpose of this study was to achieve a better understanding of pituitary growth by correlating pituitary weight, cell size and cell number in animals of different ages.

Materials and methods. Pregnant Sprague-Dawley rats were monitored closely to determine precise time of delivery. Pituitaries were removed from 1-, 10-, 25-, and 120-day-old female rats and weighed on a Cahn electrobalance or a Mettler analytical balance after removal of the posterior lobes.

Pituitary cell sizes were determined by measurement of intact cells prepared by enzymatic digestion[7]. Pituitaries from 1-, 10-, 25-, and 120-day-old rats were digested in 0.05% Pronase (Calbiochem) for 1, 1.5, 2, and 3 h respectively. Adult pituitaries were minced slightly prior to digestion. The diameter of 100–200 cells from each animal was determined with an ocular micrometer in a Bausch and Lomb microscope. Cell sizes were recorded to the nearest whole μm.

Cell number was determined by homogenization of glands in 0.25–0.5 ml cold TMK medium (0.01 M Tris, 1.5 mM $MgCl_2$, 10 mM KCL, pH 7.2)[7]. For 1- and 10-day-old animals, 5–10 pituitaries were pooled. Nuclear counts were obtained using a hemocytometer. Nuclei isolated in the manner described account for 94% of total pituitary DNA[7]. Nuclear counts are assumed to be equivalent to cell counts[2]. Analysis of variance and the Duncan range test were used to verify significance of data.

Results and discussion. Pituitary weight increased more than 3-fold during the 1st 10 days, but only doubled in the next 15 days (table). Pituitaries from adult animals weighed more than 5 times those from 25-day-old animals. The number of cells increased more than 3-fold in the 1st 10 days of life, but the rate slows not quite doubling in the next 15 days (table). Related patterns in mitotic activity and synthesis of DNA have been observed in pituitaries of immature rats[5,6]. Cell numbers increased more than 3 times between day 25 and day 120 to a mean value of 6.09 ± 0.31 million per pituitary.

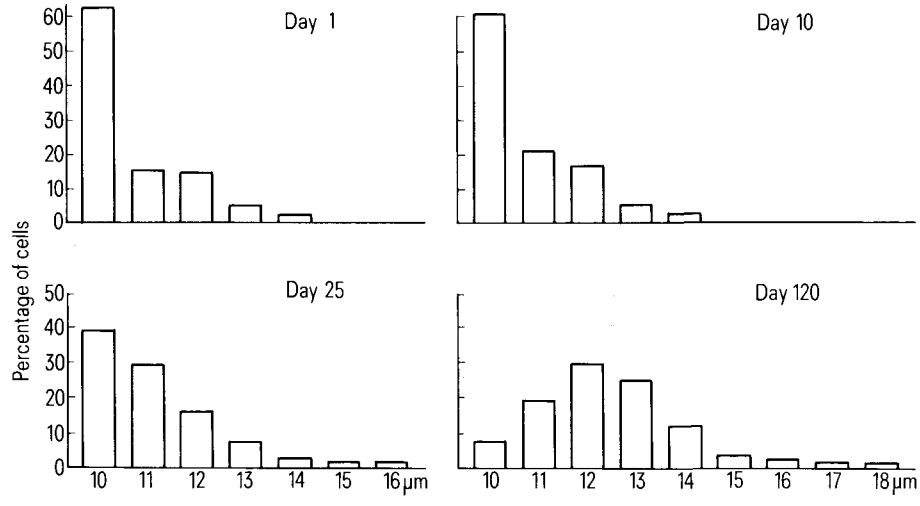
Pituitary cellular diameters of 1- and 10-day-old animals ranged from 10–14 μm with the most being at the small end of the range (figure). There was no significant increase in the number of large cells during the first 10 days. The increase in pituitary weight during this period must be mainly the result of hyperplasia and not hypertrophic changes. At day 25, there was a slight increase over day 10 ($p < 0.01$) in cell size. Although the cells which measured 10 μm remained the most numerous, there was a smaller proportion in this group than in the 10-day-old animal. A small percentage of cells was 15 and 16 μm in diameter, larger than any cells encountered in the younger animals. Therefore, in this interval, hyperplasia remained the dominant element in pituitary growth.

There was a further increase in the number of large cells in adult animals ($p < 0.01$). A large fraction of cells were 12 or 13 μm in diameter and some cells were 17 or 18 μm. Cell size measurements were used in cell volume calculations.

Pituitary weight and cell numbers in different age animals

Age (days)	Pituitary weight (mg)	cell numbers ($\times/10^{-6}$)
1	0.26±0.04	0.233±0.007
10	0.91±0.06	0.879±0.07
25	1.8±0.1	1.660±0.04
120	10.57±0.51	6.09 ±0.31

Results are the mean± SEM of 5–10 determinations. All values significantly different at $p < 0.01$.



Percentages of different size pituitary cells from different aged rats. Results are from 5–7 determinations.

Besause the cells were spherical in suspension, the volumes were calculated using the formula for sphere volume. Cell volumes ranged from 523 μm^3 to 3052 μm^3. The mean cell volume of pituitary cells was 742 μm^3 and 1105 μm^3 for 25- and 120-day-old rats, respectively. The adult cells showed an increase in volume 1.6 times that of cells of 25-day-old animals. Total volume of all cells in the pituitaries was calculated. The volume for adult pituitaries was 5.4 times the volume of 25-day-old rats. This value is in excellent agreement with the 5.8 times increase in pituitary weight between 25- and 120-days-of-age. Much of the weight increase between days 25 and 120 is the result of cellular hyperplasia. Definite cellular hypertrophy was demonstrated by the presence of 17 and 18 µm diameter cells which were not present in younger animals. The greater proportion of cells in the 12–13 µm range may have also developed by hypertrophic changes of smaller cells. An alternate explanation for this change in size distribution is that cells which are inherently large divide more rapidly than small cells, resulting in a significant population of large cells being formed by hyperplasia. Hypertrophy has a role, but the degree of involvement cannot be determined by this data.

Hyperplasia is the only mechanism of growth apparent in the preweanling rat pituitary. The high rate of DNA synthesis and mitosis during this period is consistent with this observation, but do not of themselves exclude hypertrophy[5,6]. Between days 10 and 25 there is a slowing in cellular multiplication and a slight shift to a population of larger cells. This may well be a critical period in postnatal development. The mean weight of rat hepatocytes increases sharply between days 21 and 41[3]. In addition, between days 21 and 103 the number of rat hepatocytes increases 3.2 times[3], while pituitary cells increase 3.6 times between days 25 and 120. It is not known if similar mechanisms control growth in these 2 organs. However, both liver and pituitary possess receptors for estrogen[7,8]. In addition, estrogenic hormones increase DNA synthesis and weight in both organs[9–11].

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Protective effect of calcium against the verapamil-induced inhibition of ionophore-mediated calcium translocation[1]

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Summary. The inhibitory effect of verapamil on A23187-mediated calcium translocation is antagonized in a competitive manner by increasing concentrations of calcium.

Organic calcium-antagonists such as verapamil, nifedipine and suloctidil are widely used both in the management of patients with cardio-vascular diseases and as a tool to interfere with calcium influx into living cells[2]. The effect of these drugs upon cellular calcium metabolism could be due to interference with native ionophoretic systems mediating the transport of calcium across the plasma membrane. Indeed, in an artificial system for the study of ionophoresis, verapamil and other organic calcium-antagonists were found to inhibit the translocation of calcium mediated by the antibiotic ionophore A23187[3]. The present study reveals that, as observed in intact cells, calcium itself protects against the verapamil-induced inhibition of A23187-mediated calcium translocation.

A small volume (0.2 ml) of Hepes buffer (25 mM; pH 7.0) containing Na^+ 123, K^+ 5 and Cl^- 120 mEq/l and ^{45}calcium (10 µCi/ml), with or without ^{40}calcium (1.0 mM) was vigorously mixed for 1 min at room temperature with an equal volume of a mixture of toluene-butanol (7/3, v/v) containing, as required, the ionophore A23187 (Eli Lilly, Indianapolis) and verapamil (Knoll, Ludwigshafen). The immiscible supernatant phase was then examined for its radioactive content.

The data illustrated in the table indicate that, at 2 calcium concentrations in the initial aqueous phase (8.0 µM and 1.0 mM), the amount of calcium eventually translocated in the organic phase increased as the concentration of A23187 was raised from 5 to 50 µM and, at a fixed concentration of the ionophore, decreased as the concentration of verapamil was raised to 2.5 and 5.0 mM. The dose-action relationship for the effect of increasing concentrations of A23187 (A, expressed as µM) upon calcium translocation (T, expressed as nM) was compatible with the equation $[T]=a[A]^b$ (figure, left panel), in which the factors a and b varied as a function of the concentration of calcium in the initial aqueous phase. At initial calcium concentrations of 8 µM and 1.0 mM, respectively, the factor a corresponded to a translocation of calcium, provoked by A23187 1.0 µM, of

A23187 (µM)	Verapamil (mM)	Calcium in aqueous phase 8.0 µM	1.0 mM
5	–	0.007 ± 0.001	0.396 ± 0.021
25	–	0.172 ± 0.004	4.789 ± 0.102
50	–	0.517 ± 0.029	12.565 ± 0.064
50	2.5	0.238 ± 0.010	8.603 ± 0.408
50	5.0	0.072 ± 0.001	3.902 ± 0.282

Mean values (± SEM; n = 3) for the concentration of calcium (µM) in the immiscible organic phase.