# Compensatory hypertrophy of the Leydig cells in hemiorchidectomized adult rats[1]

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*Summary.* Hemiorchidectomy in adult rats results in hypertrophy of the Leydig cells in the remaining testis. The other parts of the testis appear to be unaffected.

Hemiorchidectomy in prepubertal and pubertal rats is generally claimed to result in a compensatory hypertrophy of the remaining testis, mediated by an increase in the levels of FSH and LH[2,3]. However, hemiorchidectomy in adult rats does not influence the weight of the remaining testis[4-6], and serum concentrations of FSH, LH and testosterone and reported to be unaffected[5]. In contrast others have reported increased levels of LH up to 30 days after hemiorchidectomy in adult rats[4].

We have previously shown that the concentration of testosterone in the remaining testis after hemiorchidectomy in adult rats was highly increased, but plasma testosterone concentration and the number of Leydig cells per testis were unaffected[6]. These results indicated a compensatory hyperfunction in the Leydig cells in the remaining testis. Against this background we found it interesting to investigate whether any morphological signs of hyperfunction can be observed in the testes in hemiorchidectomized animals.

*Materials and methods.* 32 adult (4–5 months) Sprague-Dawley rats were used (Anticimex, Sweden). In 16 rats hemiorchidectomy was performed on the left side according to a method previously described[6]. The remaining, non-operated rats, were used as controls. The operated and the control rats were kept under normal laboratory conditions. Daylight was provided during 12 h per day. 4 or 8 weeks after the operations, operated and controls animals were killed and their testes were fixed by vascular perfusion via the aorta using a solution containing 4% glutaraldehyde, 3% formaldehyde and 0.05% picric acid in 50 mM cacodylate buffer[7], pH 7.2–7.4, 1350–1400 mOsm/kg, 20 °C. After the perfusion the testes were weighed and cut into pieces approximately 1 $mm^3$ in size. Five such pieces per testis were postfixed in 1% $OsO_4$, dehydrated, and embedded in Epon (for details see Bergh and Helander[8]). Testicular morphology was studied in 1-μm-thick ultramicrotome sections. Using a square lattice (121 points) mounted in the eye piece of a light microscope the following morphometric data were obtained; a) volume density of the tubules i.e. the proportion of the total testicular volume occupied by the tubules (measured at magnification × 100). b) volume density of Leydig cells i.e. the porportion of the total testicular volume occupied by Leydig cells (measured at magnification × 500). c) Tubular diameter (measured at magnification × 250). d) Total tubular length per testis. e) Total Leydig mass per testis. f) The estimated volume of an average Leydig cell. This was calculated as the ratio between the volume density and the numerical density of Leydig cells (the numerical density of Leydig cells was estimated by counting nuclear profiles and the volume density of Leydig cell nuclei), using the formula of Weibel et al.[9], assuming that Leydig cell nuclei are spherical (measured at magnification × 1000 using oil immersion optics). g) The number of Leydig cells per testis. This was calculated from the total Leydig cell mass and the average Leydig cell volume. The number of Leydig cells per testis obtained by this morphometric method is about the same as that found by others after hemocytometer counting of Leydig cells in testicular homogenates[10]. h) Leydig cell profile area. The area of 20 randomly choosen Leydig cell profiles containing nuclei was measured at magnification × 1000 using oil immersion optics. A square lattice giving 2.2 test points per 10 $\mu m^2$ was used. The methods used to obtain the morphometric data have previously been described[8,11].

*Results.* There was no difference in body weights between operated and control rats. The results of the morphometric measurements are summarized in tables 1 and 2.

Table 1. Morphometric data on hemiorchidectomized adult rats, 4 and 8 weeks after the operation

| | 4 weeks | | 8 weeks | |
|---|---|---|---|---|
| | Orchidectomized (n = 8) | Control (n = 8) | Orchidectomized (n = 8) | Control (n = 8) |
| Testicular weight (g) | 1.88 ± 0.15 | 1.89 ± 0.08 | 2.01 ± 0.18 | 1.96 ± 0.12 |
| Tubular diameter (μm) | 308 ± 17 | 304 ± 16 | 304 ± 15 | 305 ± 7 |
| Total tubular lenght per testis (m) | 22.3 ± 3.0 | 24.1 ± 2.4 | 23.9 ± 2.6 | 24.0 ± 2.9 |
| Volume density of tubules (%) | 87.8 ± 1.4 | 88.2 ± 0.7 | 89.5 ± 0.6 | 89.0 ± 0.9 |

Values are means ± SD.

Table 2. Morphometric data on the Leydig cells in hemiorchidectomized adult rats, 4 and 8 weeks after the operation

| | 4 weeks | | 8 weeks | |
|---|---|---|---|---|
| | Orchidectomized (n = 8) | Control (n = 8) | Orchidectomized (n = 8) | Control (n = 8) |
| Volume density of Leydig cells (%) | 1.91 ± 0.25* | 1.33 ± 0.12 | 1.80 ± 0.23* | 1.37 ± 0.17 |
| Total Leydig cell mass per testis (mg) | 36.2 ± 7.0* | 25.7 ± 2.8 | 36.1 ± 6.3* | 26.9 ± 4.0 |
| Leydig cell profile area ($\mu m^2$) | 130 ± 12* | 109 ± 7 | 127 ± 10* | 104 ± 8 |
| Estimated Leydig cell size ($\mu m^3$) | 1646 ± 225* | 1259 ± 208 | 1764 ± 251* | 1332 ± 180 |
| Total number of Leydig cells per testis (× $10^6$) | 22 ± 4 | 21 ± 4 | 21 ± 4 | 21 ± 3 |

Values are means ± SD. *Significantly larger than in control tests according to Wilcoxon-Mann-Whitney U-test for unpaired samples ($p < 0.01$).

*Discussion.* The present study demonstrated that hemiorchidectomy in adult rats results in approximately a 34% increase in the total Leydig cell mass in the remaining testis. The average Leydig cell profile area was larger in hemiorchidectomized rats than in controls. If these values (8 weeks) are compared on a volume basis ($127/104^{3/2} = 1.35$ it appears that the average Leydig cell in the hemiorchidectomized rats was about 35% larger than in controls. Average Leydig cell volume estimated using in independent method was about 32% larger than in controls. It thus appear that the increased total Leydig mass in the remaining testis after hemiorchidectomy is mainly due to a corresponding hypertrophy, and not hyperplasia, in the Leydig cells. This findings are in line with the observations of an increased Leydig cell nuclear diameter in partly castrated bulls[11] and a very moderate Leydig cell hyperplasia (5–7%) in hemiorchidectomized pubertal and adult rats[6,13]. Moreover, it has previously been demonstrated that there is a strong positive correlation between average Leydig cell size, and not Leydig cell number, and plasma testosterone levels in seasonally breeding animals[14].

Plasma testosterone concentration is maintained at normal values in hemiorchidectomized adult rats[4–6], but testis testosterone concentration is reported to be doubled[6]. These findings suggest that the Leydig cells in the remaining testis after hemiorchidectomy may produce twice as much testosterone as those in a normal testis. However, since total Leydig cell mass was only increased about 34% in these testes, it is likely that the average Leydig cell, apart from being large, also has a qualitatively increased capacity to produce testosterone. The mechanism behind the Leydig cell hypertrophy is unknown but it may be related to changes in the LH levels after the operation as suggested by Howland and Skinner[4]. Apart from the Leydig cell hypertrophy no other compensatory phenomena were noted in the remaining testes in hemiorchitectomized rats. These findings fit well with the general observation that hemiorchidectomy in adult rats does not influence the plasma concentration of FSH[4,5].

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## Effect of des-Tyr$^1$-$\gamma$-endorphin on serotonin metabolism in rat brain regions

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*Summary.* Intracerebroventicular (i.c.v.) administration of des-Tyr$^1$-$\gamma$-endorphin (0.1 and 1.0 µg) caused a decrease of the serotonin (5-HT) concentration of the hippocampus. The concentration of 5-HIAA and the pargyline-induced alterations in 5-HT and 5-HIAA were not affected. No effects were noticed in other brain regions.

The $\beta$-lipotropin ($\beta$-LPH) derivative $\beta$-endorphin ($\beta$-$LPH_{61-91}$), which possesses opiate activities, affects, like narcotic drugs, serotonin (5-HT) metabolism in rat brain[4–6]. The $\beta$-endorphin fragment des-Tyr$^1$-$\gamma$-endorphin ($\beta$-$LPH_{62-77}$, DT$\gamma$E), which is devoid of opiate activity, induces neuroleptic-like effects in rats[7] and has an anti-psychotic action in schizophrenic patients[8,9]. Since both serotonergic and dopaminergic receptors in the brain may be involved in the mechanism of action of neuroleptics[10], we studied the influence of DT$\gamma$E on 5-HT metabolism in rat brain regions.

*Methods.* Male Wistar rats (Centraal Proefdieren Bedrijf TNO, Zeist, The Netherlands) weighing 175–205 g were used. They were housed under standard conditions (22 °C, lights on from 5.00 h to 19.00 h) and had access to food and water ad libitum. The rats received a polyethylene cannula in the lateral ventricle 9 days before the day of experimentation and were housed in single cages afterwards. The rats were fasted for 20 h before being decapitated. In the 1st experiment rats received either placebo (1 µl saline) or 1.0 µg DT$\gamma$E in 1 µl saline intracerebroventricularly (i.c.v.). 30 min later 8 rats of each group were decapitated; the other 16 rats of each group received either pargyline (150 mg/kg; Aldrich) or vehicle (saline) i.p. and were subsequently decapitated 30 min later, i.e. 60 min after i.c.v. treatment. In a 2nd experiment the effect of higher and lower doses of DT$\gamma$E on hippocampal 5-HT concentration was investigated. 4 groups of 8 rats received 1 µl saline or 0.01, 0.1 or 10.0 µg DT$\gamma$E in 1 µl saline i.c.v. 30 min later all rats received 0.5 ml saline i.p., in order to create experimental conditions identical to those of the 1st experiment, and they were then decapitated 60 min following i.c.v. treatment. After decapitation the brains were rapidly removed and dissected according to Gispen et al.[11] on an ice-chilled glass plate. 5-HT and 5-hydroxy 3-indole acetic acid (5-HIAA) were assayed in the brain parts (indicated in table 1) according to Curzon and Green[12]. Concentrations are expressed as ng per mg tissue and given as means ± SEM. Student's t-test was used for statistical analysis of the data.

*Results.* The results of the 1st experiment are shown in tables 1 (5-HT) and 2 (5-HIAA). 60 min after i.c.v. DT$\gamma$E administration the 5-HT concentration of the hippocampus was decreased as compared with that after saline treatment. No differences were found in 5-HT concentration in the other brain parts and in 5-HIAA concentration in any of the brain regions. Pargyline administration resulted in an increase in 5-HT and decrease in 5-HIAA concentrations in all regions. There were no significant effects of peptide treatment on the pargyline-induced alterations of 5-HT and 5-HIAA concentrations. In the 2nd experiment it was found that also a dose of 0.1 µg DT$\gamma$E caused a decrease in hippocampal 5-HT concentration (728 ± 21 ng/g vs 809 ± 19 ng/g