Effect of immunization of male rabbits against androstenedione

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Summary. After active immunization of male rabbits against androstenedione and with increasing antibody titre the concentration of androstenedione and testosterone in peripheral blood increased more than 1000-fold resp. 20-fold above values of control animals. On histological examination of the testes a marked Leydig's cell hyperplasia was found, though there was no difference in testis weight.

The radioimmunoassay of steroid hormones requires specific antisera, which are produced by active immunization of e.g. rabbits against a steroid-protein-conjugate. For the specificity of the antibody the position of the steroid molecule where the protein is conjugated is important.

Nieschlag et al.[1-3] reported on the effects of active immunization of male rabbits against testosterone and androstenedione respectively, which were conjugated to bovine serum albumin through position 3 of the steroid molecule.

Using a similar immunization scheme but a different antigen (androstenedione-11α-BSA) we examined in actively immunized male rabbits the circulating levels of androstenedione and testosterone as well as the histological alterations of the testes[4].

Material and methods. Eight 8-week-old male Chinchilla rabbits were immunized with 50 resp. 75 µg of 4-androstene-11α-ol-3,17-dione-hemisuccinate-BSA (Steraloids, USA) according to the multiple site method of Vaitukaitis et al.[5].

4 rabbits with antibody titres below $^1/_{100}$ received a booster injection after 8 weeks and all rabbits after 12 weeks. 2 rabbits with high antibody titres ($> {}^1/_{2000}$) 20 weeks after the immunization were again boostered i.p. in the 25th week. 3 male rabbits of the same age served as a control group.

Blood samples were taken weekly from an ear vein and analyzed for antibody titre and the concentration of androstenedione and testosterone.

For the determination of the antibody titre and in the androstenedione-radioimmunoassay [1,2,6,7-^{3}H] androst-4-ene-3,17-dione was used as the labelled antigen. After incubation at 4 °C overnight, the bound and free fraction were separated using dextran-coated charcoal.

The antibody titre was defined as that dilution of the antiserum, which displayed 50% binding of the labelled antigen (10,000 dpm; 10 pg of steroid).

The radioimmunoassays for androstenedione and testosterone were performed according to the method of Nieschlag and Loriaux[6].

Results. 2 rabbits showed a very weak immune response with antibody titres between $^1/_{70}$ and $^1/_{320}$. 4 rabbits had final antibody titres between $^1/_{460}$ and $^1/_{1300}$. Only 2 of the 8 immunized rabbits reached high antibody titres ($^1/_{3800}$ and $^1/_{14000}$) after the 2nd i.p. booster injection.

The antisera displayed the following cross reactions with other steroid hormones: androstenedione: 100%, 11α-OH-androstenedione: 100%, testosterone: 0.8-4.4%, 5α-dihydrotestosterone: 0-0.4%, corticosteroids, progesterone, estrogens: $< 0.1\%$.

The standard curve, plotted on a semilogarithmic scale, showed linearity between 25 and 500 pg of unlabelled androstenedione with 34.6 resp. 88.5% displacement of the labelled antigen. With increasing antibody titres the concentration of androstenedione and testosterone in the peripheral blood of the immunized male rabbits increased several fold above values found in the control group (table).

There were no statistically significant differences between the weights of testes and epididymis of rabbits in the control and experimental group. On histological examination of the testes however, we found in the immunized rabbits a significantly increased number of Leydig's cells with enlarged nuclei in comparison with the controls. The testes of the immunized rabbits showed more sudanophilic granula in the Leydig's cells than those of control animals.

Discussion. According to the low percentage of cross reaction with other steroid hormones - except 11α-OH-androstenedione, which is present only in very low concentration in the peripheral blood of cows under physiological conditions - the antisera raised are well suited for their use in a radioimmunoassay of androstenedione in bovine blood without preceding chromatography, as blood samples esti-

Antibody titre (1/...), androstenedione (Δ4) - and testosterone (T) - concentration (ng/ml) in male rabbits after active immunization against androstenedione (– = not measured)

Rabbit No.	Weeks after 1st immunization																					
	8			13			15			18			20			27			28		
	Titer	Δ4	T	Titer	Δ4	T	Titer	Δ4	T	Titer	Δ4	T	Titer	Δ4	T	Titer	Δ4	T	Titer	Δ4	T
Immunized																					
1	80	–	–	80	–	–	200	–	–	320	–	–	–	–	–	–	–	–	–	–	–
2	40	–	–	80	–	–	80	–	–	70	–	–	–	–	–	–	–	–	–	–	–
3	70	–	0	100	–	10	400	–	28	460	–	30	460	–	30	–	–	–	–	–	–
4	1200	30	12	500	10	10	640	56	20	720	17	18	580	–	–	–	–	–	–	–	–
5	130	20	13	270	78	10	920	126	–	1200	248	34	2440	308	24	2840	116	12	3800	210	22
6	40	0	0	50	40	12	620	100	–	1030	90	20	1300	96	22	–	–	–	–	–	–
7	680	172	50	260	50	30	760	152	–	2040	164	62	1020	–	–	–	–	–	–	–	–
8	2240	194	20	180	8	0	1420	132	–	2200	68	26	2440	–	–	1460	–	–	14000	–	–
Control																					
9				–	–	–															
10				–	0.2	2.6															
11				–	0.1	0.6															

mated before and after Sephadex-LH 20 chromatography gave identical results[7].

The biological effects of the active immunization of male rabbits against androstenedione differ in some respects from the results of Nieschlag et al.[2], as we found a significant increase of the concentration of androstenedione and testosterone in peripheral blood, as well as Leydig's cell hyperplasia, but no significantly increased testicular weight. An explanation for this difference may be the different specificity of the antibody as the antigens were conjugated in a different position of the steroid molecule to the protein.

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Hemicastration-induced changes in the electrophoretic pattern of some enzymes in the brain of the skink, *Mabuya carinata*

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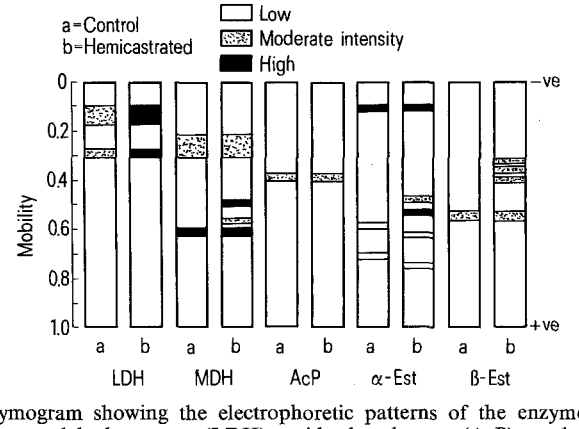
Summary. Hemicastration in the skink induces change in the electrophoretic pattern of some enzymes like LDH, MDH, acid phosphatase and esterases.

Hemicastration-induced compensatory hypertrophy of the contralateral gonad is said to be due to the removal of gonadal hormone feedback check on the hypophysis and consequent increased release and/or utilization of hypophysial gonadotrophins[2,3]. This hemicastration-induced compensatory hypertrophy is blocked by gonadal hormones like estrogen, progesterone and testosterone, indicating that the former explanation may be true[4–6]. We report here some interesting information about the electrophoretic patterns of some enzymes in the brain of the hemicastrated male skink.

Materials and methods. Sexually mature male skinks, weighing 18–25 g, collected in and around Mysore city during the month of September, were hemicastrated by surgical removal of the right testis. Sham-operated controls were also used. Each group contained 5 animals. On the 21st day of hemicastration, the animals were autopsied; the brain was dissected out and immediately homogenized in 0.1 M phosphate buffer pH 7.0 (1:2.5 w/v) using a tissue homogeniser at 5 °C. The homogenate was centrifuged at 3000 rpm at 5 °C for 1 h in an MSE refrigerated centrifuge. Protein concentration in the homogenate was estimated by the method of Lowry et al.[7]. About 150 µg of protein from this sample was layered on the gel in 40% sucrose to carry out polyacrylamide disc gel electrophoresis as described by Davis[8]. Tris-glycine buffer at pH 8.5 was used in the run at 5 °C, applying a current of 4 mA per gel. The gels were stained using suitable procedures[9,10] for acid phosphatase (AcP), α- and β-esterases (Est), lactate dehydrogenase (LDH) and malate dehydrogenase (MDH).

Results and discussion. Electrophoretic patterns for various enzymes and their mobilities are given in the figure. There is no difference in the electrophoretic patterns of LDH bands between the hemicastrated animals and sham-operated controls. But an increase in the total activity of both the bands was observed in the hemicastrated skinks. Acid phosphatase does not show any difference in the number of bands or their activity between the control and experimental groups. The electrophoretic pattern with reference to MDH and both esterases was altered, however. All 3 enzymes show new bands in addition to the bands present in the enzymes from the brains of the control animals. 2 new additional MDH-bands appeared, and 3 new additional β-esterase bands were found. The α-esterase even has 4 new cationic bands in hemicastrated animals, and 2 cationic bands found in the controls disappear.

The results indicate an overall increase in the total activity as also in the number of bands representing isozymic patterns in 3 enzymes. The oxidative enzymes have been reported to be involved in lipid metabolism and carbohydrate metabolism. Ascribing any definite function to the hydrolytic enzymes, especially esterases, in any tissue is not possible as their occurrence is ubiquitous. But it can be said



Zymogram showing the electrophoretic patterns of the enzymes lactate dehydrogenase (LDH), acid phosphatase (AcP), malate dehydrogenase (MDH), α- and β-esterases (Est) in the brain of normal and hemicastrated skink, *Mabuya carinata*.