اثر هورمونهای HCG، تستوسترون و عصاره هیپوفیز بر پینه شست و پوست بدن در وزغ‌های نر بالغ گونه (Bufo viridis)

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چکیده

در این بررسی اثرات ماکروسکوپیک و بافتی گونا دو تروپینها و تستوسترون بـر روی پـینه شست و پوست در وزغ‌های نر بالغ گونه (Bufo viridis) مورد مطالعه قرار گرفت. مجموعاً سه سری آزمایش بر روی وزغ‌های نر سالم انجام شد. در سری اول آزمایش‌ها به حیوانات تجربی روزانه دو غده هیپوفیز وزغ ماده بمدت سه روز تزریق شد. در سری دوم از HCG به مقادیر ۲۵، ۵۰ و ۱۰۰ واحد استفاده شد. و در سومین سری از آزمایش‌ها روزانه تستوسترون به مقادیر ۵،۲/۵،۰/۵ و ۱۰ میکروگرم به ازای هر گرم وزن بدن بمدت ۱۲ روز به حیوانات تجربی تزریق گردید. نتایج حاصل از این تجربیات نشان میدهد که پینه شست و پوست در حیوانات تجربی نه‌تنها تیره‌تر شده است بلکه ضخامت اپی‌درم و تعداد غدد درمی و پاپیلای اپی درمی نیز در آنها افزایش یافته است. نظیر اینگونه نتایج پس از تزریق مقادیر فوق‌الذکر از تستوسترون به حیوانات نر اخته شده نیز بدست آمد. بنظر میآید که اثر تستوسترون و گونا دوتروپین‌ها در نمو پینه و شست و تغییرات پوستی که از علائم رفتار جنسی در اینگونه دوزیستان است به علّت وجود رسپتورهای آندوروژنی در اندامها مذکور می‌باشد.

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THE EFFECTS OF PITUITARY GLAND, HCG AND TESTOSTERONE ON THE THUMB PADS AND THE SKIN OF ADULT MALE TOADS (BUFO VIRIDIS)

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Abstract

Macroscopic and histological observation of the skin and the thumb pads on adult male toads weighted 27-30 gr were studied under defined conditions (Room temperature

20-25°C and photoperiod of 12L: 12D). Groups of animals received intraperitoneally injection of whole female pituitary gland and different doses (25, 50, 100, 200 IU) of HCG and (0.5, 2.0, 5, 10 μg/g B.W.) of testosterone. Studies on experimental animals showed increase in thickness, pigmentation, number of dermal glands and epidermal papillae in thumb pads and skin in all groups. Injection of different doses of testosterone in castrated males showed the same results. The effect of sexual hormones on the thumb pads and skin appear to be related to the androgen receptors in these organs which become active during reproductive period and sexual behaveiour.

Introduction

The effects of androgen administration on the development of male sexual characters have been studied by many investigators in various anurans species. It is well known that thumb pads are androgen dependent and have higher capacity to retain labelled testosterone and its metabolites such as 11-keto testosterone and androstanolone (Delrio, 1973 D´Istria, 1972). on the other hand, the administration of testosterone to young female Rana nigromaculata with supernumerary forelimb induces the development of thumb pads on the normal forelimb as well as on the supernumerary limb (Iwasawa, 1985). Although the effects of testosterone on the development of thumb pads were well known, the effects of different doses of Testosterone and gonadotropins seem quite questionable.

The present study was designed to determine the role of these factors on the thumb pads and skin.

Materials and Methods:

All experiments were performed on the adult male toads of Bufo viridis collected from suburb of Tehran with body weight 27-30g and body length 6-7.5 cm.

Room temperature was maintained at 20-25°C, and photoperiod of 12L:12D suggested by Iwasawa was used (Iwasawa, 1984). pieces of sheep liver were offered to the animals every 3 days as food. We have done three experiments on the intact toads. Groups of 3 toads were studied in each experiment. The doses of administered hormones to each toad are shown in tables 1,2,3,4. The pituitary gland used in the present study were obtained from adult female B ufo viridis and homogenized in the . 0.64% NaCl solution (Humasan, 1972). The male toads received two female pituitary glands per day for three successive days. The human chorionic gonadotropin (HCG) was obtained from L.F. SERONO S.P.A. Company. This hormone was dissolved in the 0.64% NaCl for injection.The Testosterone hormone was obtained from Aboreihan Company. The testosterone concentration was reduced by dissolving the hormone in olive oil.

24 hour after injection of female pituitary gland or HCG, and three days after injection of testosterone, the thumb pads and a piece of skin of each toads were fixed in Bouin's solution, then embedded in paraphin wax. Serial sections were cut

crossly at 6μm thickness and stained with Mayer's hematoxylin and eosin. The degree of histological changes was judged quantitatively in 10 cross sections of thumb pads and skin, the tickness of the epidermis, number of dermal glands and papillae of the thumb pads, were counted. The results were tested statistically for significance by student test and analysis of variance.

Results:

The results of histological studies of thumb pads and skin after injection of female pituitary gland. HCG ε testosterone are shown in tables 1,2,3,4 respectively. As shown is tables, the development of thumb pads and skin werenot noticeable histologically in control taods.

Generally, dermal glands in thumb pads and skin were free from secretory granules. Papillary formation wasnot clearly seen and epidermal pigments were distributed. After administration 2 and 4 pituitary glands, the thickness of epidermis and the number of epidermal papillae in thumb pads and skin increased. There was no change in the number of dermal glands in thumb pads, but in skin, after administration of 4 pituitary glands the number of glands increased (Figs 1,2). By injection of 6 pituitary glands, not only the thickness of epidermis increased in the thumb pads and skin, but also the number of dermal glands increased in the thumb pads, and some of these were filled by secretory granules and extended to the surface of the epidermis (Fig.3). Administration of 100 IU HCG, showed increase in epidermal thickness and papillae in thumb pads and skin while opposed to the thumb pads the number of the dermal glands of skin werenot significantly changed (Fig.4). Injection of 2.5 μg/gBW/day testosterone to intact animals seemed to cause an increase of epidermal thickness and papillae of thumb pads, while injection of 5-10 μg/gBW/day testosterone showed also increase in the number of dermal glands. In the animals treated with 0.5,2.5, 5μg testosterone no change were seen in the epidermal papillae of skin, but injection of 10- μg/gBW/day was effective in the number of dermal glands and epidermal thickness (Fig. 5.6). Gonadectomy in the males caused atrophic changes in thumb pads and skin, and depigmentation was observed in these tissues while after administration of testosterone, the thickness of epidermis and papillae and the number of dermal glands were increased. Maximum increase in the number of dermal glands was observed in the toads treated with 10μg testosterone. Administration of 2.5 and 5μg testosterone had no effect on the number of dermal glands (Figs 7,8).

Discussion:

Macroscopic studies showed that administration of pituitary gland, HCG and testosterone induced the development of thumb pads in this species. After administration of testosterone alone, thumb pads and skin became darker and thicker. Therefore it seems that development of the thumb pads is greatly influenced by exogenous testosterone in this species and pituitary gland and HCG have an indirect effect on this process.

Thumb pads and skin, having testosterone receptors, are the target tissues for this sex hormone (D´Istria et al, 1972). It is well known that specific characters in thumb pads and skin occurred

parallel with the dose of administered testosterone. In toads treated with 5μg testosterone, no significant changes were found in the number of dermal glands and epidermal papillae, while 10μg testosterone was effective.

It seems that the specific amount of testosterone is necessary for development of epidermis and dermal glands, presumably, range of physiologic level for each stage of development is different.

Table 1:Experimental procedure and results of t-test student developmental degree of thumb pads and skin after injection of pituitary gland. (Mean ± SD)

Groups	Thumb pad			skin		
	Thickness of epidermis (μm)	Number of dermal glands	Number of epidermal papilar	Thickness of epidermis (μm)	Number of dermal glands	Number of epidermal papillae
Control	32.58±7.06	12.5±3.03	7.7±4.19	7.96±2.34	4.7±2.26	5.8±1.75
2 pituitary glands	39.97±7.33	12.9±3.11	23.8±3.36	37.02±17.59	11.3±4.16	10.4±1.78
p	0.05	0.1	0.001	0.001	0.01	0.001
control	21.10±7.69	11.4±1.07	16.8±4.08	7.44±2.59	4.4±2.22	6.8±2.04
4 pituitary gjands	40.36±7.50	14.9±6.40	26.5±5.74	37.44±5.38	13.2±4.21	11.3±1.89
p	0.001	0.1	0.01	0.001	0.001	0.001
control	23.10±8.03	7.1±3.03	11.6±4.14	11.94±4.35	10.8±1.55	11.5±1.72
6 pituitary glands	42.056±16.21	19.8±5.01	43.7±9.88	37.54±5.59	12.2±1.40	18.1±2.64
p	0.01	0.001	0.001	0.001	0.1	0.1

Table 2:Experimental procedure and results of analysis of variance developmental degree of thumb pads and skin after injection of HCG. (Mean ± SD)

Groups	Thumb pad			skin		
	Thickness of epidermis (μm)	Number of dermal glands	Number of epdermal papillae	Thickness of epidermis (μm)	Number of dermal glands	Number of epidermal papillae
Control	39.44±9.62	15.9±4.89	16.6±3.81	12.11±3.9	10.1±2.33	12.5±3.92
25 IU HCG	59.86±18.93	17.6±4.90	18.4±5.72	43.94±20.1	12.9±3.25	16.8±6
50 IU HCG	102.24±38.57	18.2±2.94	35.9±5.59	49.66±24.7	12.4±1.9	23±2.75
100 IU HCG	105.35±25.10	22±6.68	36.4±11.32	49.83±6.96	9.4±2.55	27.2±4.13
F	16.33	2.62	22.58	12.11	4.49	22.33
P	0.001	0.2	0.001	0.001	0.001	0.001
dr	22.9	10.22	4.30	14.83	2.30	3.94

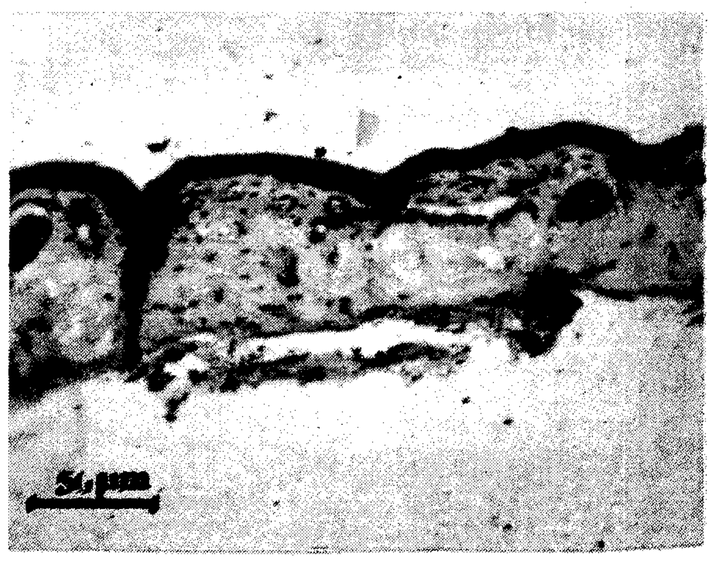
Table 3:Experimental procedure and results of analysis of variance developmental degree of thumb pads and skin after injection of testosterone. (Mean ± SD)

Groups	Thumb pad			skin		
	Thickness of epidermis (μm)	Number of dermal glands	Number of epidermal papillae	Thickness of epidermis (μm)	Number of dermal glands	Number of epidermal papillae
control	41.35±9.92	21.2±4.85	15.9±3.87	14.01±8	12.9±1.91	18.1±3.81
Testoste-rone (0.5) μg/BW/day	94.63±23.9	21.5±6.17	19.7±4.52	55.71±20.3	8.8±3.05	13.6±7.72
Testoste-rone (2.5) μg/BW/day	97.76±23.04	26.6±7.59	24.5±15.9	57.78±15.63	8.9±1.20	14.3±7.80
Testoste-rone (5) μg/BW/day	105.36±24.13	27.8±8.5	26.2±5.75	57.26±15.7	10.3±2.83	17.7±3.71

Testoste-rone (10) μg/BW/day	105.76±9.37	36.2±6.48	34.8±7.47	71.62±15	16.3±2.21	21±4.35
F	19.56	7.96	6.82	19.96	18.53	2.70
P	0.001	0.001	0.001	0.001	0.001	0.05
dr	17.47	6.26	7.84	14.14	2.12	5.27

Table 4:Experimental procedure and results of analysis of variance developmental degree of thumb pads and skin in the castrated toads after injection of testosterone. (Mean ± SD)

Groups	Thumb pad			skin		
	Thickness of epidermal (μm)	Number of dermal glands	Number of epdermal papillae	Thickness of epidermis (μm)	Number of dermal glands	Number of epidermal papillae
control	28.17±7.74	16.7±3.16	8.7±4.45	24.57±9.40	6.6±3.13	6.9±2.96
Testoste-rone (2.5) μg/BW/day	41.17±16.83	10.7±6.48	16.2±2.82	32.71±7.36	7.2±2.04	12.7±2.95
Testoste-rone (5) μg/BW/day	43.42±9.09	10.07±4.24	17.8±5.05	41.38±8.43	10±2.54	12.4±2.41
Testoste-rone (10) μg/BW/day	46.71±18.70	28.3±11.83	28±12.76	52.8±14.93	20±6.24	19.3 ±4.08
F	3.4	10.18	11.97	13.4	26.03	25.74
P	0.05	0.001	0.001	0.001	0.001	0.001
dr	12.6	5.86	6.65	9.44	3.84	2.85



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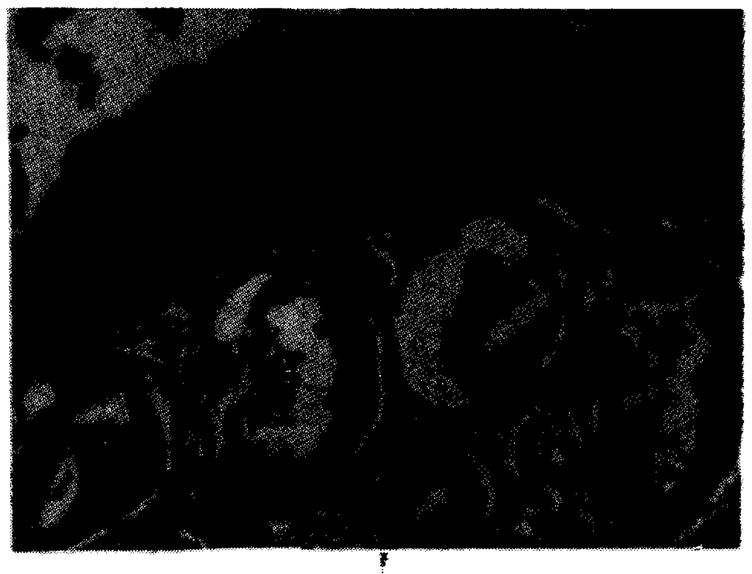

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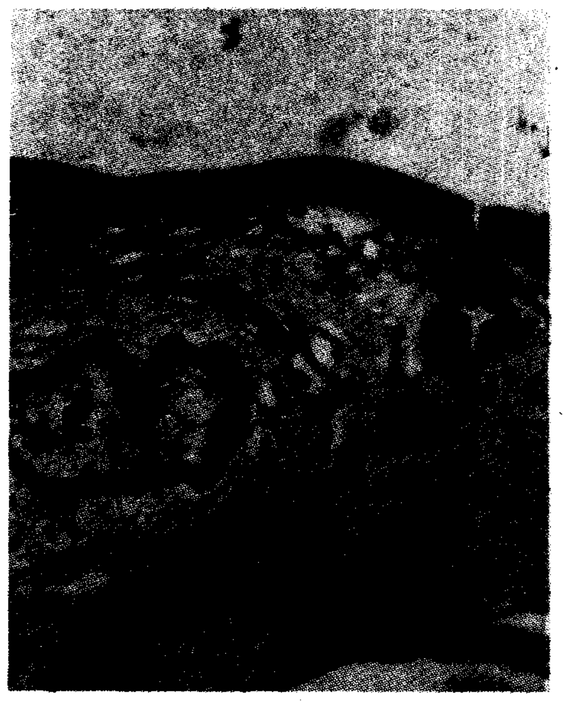
FIGS. 1-6. Cross section of thumb pad region. (1)Control. The development of the thumb pad isnot noticeable. (2-6)Epidermal thickness and the number of dermal glands increase, and papillary projections are clearly seen. (2)After 4 pituitary injection. (3) After 6 pituitary glands injection. (4)100 IU HCG- treated toad. (5) After injection of 2.5 μg/BWg/day testosterone. (6)After injection of 5 μg/BWg/day testosterone. e:epidermis g:dermal gland p:papillae



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FIGS. 7.8. Cross sections of thumb pad region in castrated toads. (7) After castration. (8)After injection of 10 μg/BWg/day testosterone to castrated toad.

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