EFFECT OF GRADED DOSES OF TESTOSTERONE ON THE WEIGHT AND THE PROTEIN CONTENT OF ACCESSORY SEX ORGANS AND SKIN OF OLD ALBINO RATS

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Summary: Graded doses of testosterone propionate were administered to intact old albino rats, and weight of sex organs, levator ani muscle and total protein content of skin and accessory sex organs were determined. It was observed that at certain dosage level the weights of seminal vesicle and levator ani muscle were reduced. The protein content of skin, seminal vesicle and levator ani muscle was increased by the administration of 25 μg dose of TP while at higher dosage level it was reduced. Administration of male sex hormone did not alter the water content of skin.

INTRODUCTION

It has been reported by different investigators that sex hormones produce a definite enhancement of rate of mitosis in skin (1,6). Allen (1) showed that testosterone stimulates mitosis in the mouse epidermis. In an experiment on male rats, castration reduced the incidence of mitosis and implantation of testosterone increased it (6). Montagna *et al* (11) first conducted a clinico-pharmacological study to assess the possibility of reversing the geriatric changes of human epidermis by local application of testosterone propionate (TP). In their experiment they found that histological changes induced by aging were restored within 6 weeks following local application of 1% TP. It is interesting to note that in paraffin section of aged untreated skin, cells of epidermis were deformed whereas in the skin of young adult and the skin of steroid treated aged subject, epidermal cells presented a healthy appearance. In the present investigation experiments were conducted to study the effect of systemic administration of testosterone on the water and protein content of skin. It is not known whether in old age the anabolic and the androgenic responses to testosterone are similar to those in young rats. Therefore, overall androgenic and anabolic responses to graded doses of testosterone were also studied.

MATERIALS AND METHODS

Old albino rats (3 years to 3½ years) obtained from Central Drug Research Institute, Lucknow, were used in this investigation. A total of thirty rats were equally divided into five groups. Group I served as control. Rest of the groups *i.e.* II, III, IV and V were treated with 25, 50, 500, and 1000 μg of testosterone propionate (TP) respectively, everyday for 7 days.

The animals were sacrificed 24 hr after the last injection. The seminal vesicle (SV), coagulating gland (CG), ventral prostate (VP) and levator ani muscle (LA) were dissected out and weighed to the nearest of 0·1 *mg*. Water content of skin, SV and LA was estimated from the difference between the weight of the fresh tissue and its dry weight. Total protein

content of different tissues was estimated by the method of Lowry *et al* (8) using Carl Zeiss Spectrophotometer (Spekol).

The significance of differences between groups was calculated by Student's "t" test.

RESULTS

The data are summarised in Table I.

Protein and Water Content of Skin

A dose of 25 μg of TP produced significant increase in the protein content of skin. The protein content was significantly reduced at 50 μg and 500 μg dosage level, while a dose of 1000 μg did not produce any change. However, the protein content of the skin was minimum at 50 μg and a gradual increase was noted with the increase in dose of TP although the value never exceeded the control level. The water content of the skin did not signficantly alter throughout the experiments.

Weight of Coagulating Gland (CG)

At 25 μg and 50 μg dosage level, the weight of CG was significantly reduced by TP. But the degree of reduction of CG weight was more at 35 μg dosage level ($P < 0{\cdot}001$) than at 50 μg dosage level ($P < 0{\cdot}002$). A dose of 500 μg of TP produced a slight increase in the weight of coagulating gland but the result was not statistically significant. At 1000 μg dosage level, the weight of CG was increased and the result was statistically significant. It is thus apparent from the response, that at low dose, the weight of CG was definitely decreased, and with gradual increase in the dose of TP a point of equilibrium was reached and finally at still higher dose a significant increase in CG weight was noted.

Weight and Protein content of Seminal Vesicle (SV)

TP induced changes in weight of SV were almost similar to those of CG, except that at 500 μg dosage level statistically significant decrease of SV weight was noted ($P < 0{\cdot}001$). Like CG, statistically significant increase of SV weight was produced by 1000 μg of TP. It is interesting to note that 25 μg of TP significantly increased the protein content of SV, but reduced the weight of the organ. At 50 and 500 μg, TP failed to produce any sifinificant change in protein content while at 1000 μg, a significant reduction in the protein content and an increase in the weight of the organ were noted. Thus it is apparent from the data that TP-induced alteration of weight and protein content are two independent phenomenon.

Weight and Protein Content of Levator Ani Muscle (LA)

The weight of LA was reduced at all the dosage levels of TP although the result was statistically significant at 50 and 1000 μg dosage levels, only. The protein content of LA was significantly increased by 25 μg of TP while significant decrease was produced by all other

dosages of TP. As in SV, the effects of TP on the protein content and weight of LA appear to be independent of each other.

Weight of Ventral Prostate (VP)

TP treatment did not produce any significant change in the weight of VP.

DISSCUSSION

The most salient feature of the data obtained in the present study is the paradoxical inhibitory effect of TP in old albino rats. The data presented in this experiment suggest that aging process is one of the determining factors for response to male sex hormone. Such age dependent response to female sex hormone has already been reported by Carter (4) and Ebling (5) who demonstrated that oestrogen did not stimulate epidermal mitosis in the adult rat although it appears to stimulate epidermal mitosis in immature rat (2,3,5). TP-induced ponderal changes in organs under study presented three different patterns viz. reduction of organ weight in low doses and gradual shift towards an increase in organ weight with the increase in dose of TP as observed with CG and SV. The second type of change was seen with levator ani muscle where TP produced a persistent decrease in the weight of the muscle and lastly ventral prostate of old albino rats was completely refractory to the action of the androgen. However, the protein content of skin, SV and LA was uniformly reduced by TP at all dosage levels except at a dose of 25 μg.

Recently Konigsmark(7) reported a few fundamental facts of aging cells and structure. These include (i) aging due to absent or decreased cell production (ii) aging with over-growth of cells and (iii) aging with intracellular accumulation of substance. He further reported that the aging process varies from cell type to cell type and from organ to organ. It is not clearly known which particular aging process determines physiological status of different accessory sex organs. In SV and CG, TP induced reduction of weight may be due to the fact that cell production was decreased in these organs and therefore no increase in weight of these organs was noted except with very high dose of TP. Moreover, aging of these organs may be responsible for intracellular accumulation of substances and TP may act by removing the accumulated substances and thereby reduce the weight of CG and SV in low dose of TP. Although the effect of TP on LA closely resembles that on CG and SV, TP-induced reduction of the weight of LA is probably mediated via a different mechanism. Since skeletal muscle is a fixed postmitotic tissue and SV and CG consist of tissues having mitotic potential, aging process in these organs is likely to differ and the mechanism of TP-induced changes is likely to be different. Patnaik(12) has reported that nitrogen, potassium and phosphorus contents of rat skeletal muscle decrease significantly with age when compared with those of liver, a mitotic potential tissue. It has been well established that TP causes retention of nitrogen, sodium and phosphorus. Thus the aging process in LA produces a metabolic change which is anti-anabolic in character

TABLE I : *Effect of graded doses of testosterone on weight and protein content of accessory sex organs and skin of old albino rats.*

Testosterone µg/day/rat.	*Weight of C.G. (mg. ±S.E.)*	*Weight of S V (mg. ±S.E.)*	*Weight of V P (mg. ±S.E.)*	*Weight of L A (mg. ±S.E.)*	*Protein content of skin (mg.g ±S.E.)*	*Protein content of SV (mg.g ∓ S.E.)*	*Protein content L A mg.g/ ±S.E.)*	*% of water content of skin ±S.E.*
Control	80.9±4.23	184.7±8.07	195.3±12.59	546.5±17.85	144.4±14.13	218.5±18.72	236.0±18.24	63.25±2.4
25	32.5±5.87 (<0.001)	139.0±12.45 (<0.02)	200.3±18.84	483.5±40.5	318.8±5.08 (<0.001)	317.5±4.8 (<0.001)	349.3±17.2 (<0.002)	60.15±5.6
50	53.2±3.86 (<0.002)	143.0∓17.23 (<0.05)	166.6±17.55	373.2±3.98 (<0.001)	87.3∓7.28 (<0.01)	226.0±12.94	138.2±8.28 (<0.001)	59.87±1.23
500	93.6±10.63	129.4±3.76 (<0.001)	217.7±12.27	493.7±16.72	97.4±9.42 (<0.01)	171.6±14.46	74.7±15.26 (<0.001)	64.27±0.52
1000	104.5±4.89 (<0.01)	220.5±12.63 (<0.002)	232.1±23.01	280.3±14.79	140.0±14.72	148.3±15.46 (<0.01)	156.3±27.79 (<0.05)	64.29±6.21

C G—Coagulating Gland

S V—Seminal Vesicle

V P—Ventral Prostate.

L A—Levator Ani Muscle.

and which could not be corrected by TP. Probably this is an irreversible change induced by aging process(9) and TP was found to aggravate such change in skeletal muscle.

Unlike young rats, TP failed to produce any ponderal change of the ventral prostate of old albino rats. Our results agree favourably with Mainwarning(9) who reported that no biochemical changes occurred either in enzyme system or in fundamental cellular constituents of ventral prostate gland of mice from the age of nine months in response to testosterone stimulation. It is interesting to note that synthesis of RNA was found to be substantially diminished in the prostate gland of aged mice(10). Moreover, aging was found to be accompanied by marked change in the sedimentation profile of ribonucleo-protein particles. This was attributed to an age associated depletion in messenger RNA or to an increased susceptibility to enzymic disaggregation. Since the mechanism involving the biosynthesis of RNA is affected by the aging process, TP-induced increase in the weight of ventral prostate is not likely to take place because such a change is associated with growth of the gland *vis* a *vis* biosynthesis of RNA. Although TP is known to cause water retention in the tissue no such effect was noted in the skin of aged rats. Moreover, protein content of the skin did not show an uniform response to graded dose of TP. While the protein content was significantly increased by 25 μg of TP, no significant change was produced at 1 *mg* dosage level. Therefore, the proposed hypothesis of Montagna *et al*(11) for beneficial action of TP on the human skin could not be substantiated from the present data. But the increase in protein content at 25 μg dosage level agrees with proposed hypothesis.

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