Journal of Steroid Biochemistry, 1970, Vol. 1, pp. 349–354. Pergamon Press. Printed in Great Britain

A COMPARISON OF THE ANDROGENIC AND MYOTROPHIC ACTIVITIES OF SOME ANABOLIC STEROIDS IN THE CASTRATED RAT

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(*Received* 16 *January* 1970)

SUMMARY

Oxymetholone, oxandrolone, methandrostenolone, nandrolone phenpropionate, norbolethone, ethylestrenol, bolasterone, norethandrolone, oxymesterone, methenolone acetate, chlorotestosterone acetate, stanozolol and testosterone were compared for effects upon seminal vesicle, ventral prostate and levator ani weights in castrated rats. Linear log dose-response relationships were obtained for all compounds on all three parameters. Variations in slopes indicated non-parallelism of dose-response lines, thus preventing calculations of exact relative potencies. Relative activities are reported based upon "doubling doses" for each target organ.

INTRODUCTION

A NUMBER of anabolic steroids are currently available for clinical use. Reports have been published on animal studies with individual anabolic steroids and papers have appeared with comparative data on a few selected compounds [1–6]. However, examination of the available literature has indicated that there are significant differences in the reported relative potencies of compounds even when comparisons were made with the same standard preparation. This is perhaps not unexpected since different laboratories have used different strains and ages of animals, different periods of treatment, and have started compound administration at various intervals after castration. Relative potencies have been estimated by a variety of techniques. This has resulted in a literature replete with inconsistent data and has made the evaluation of the effects of structural modifications on activity difficult for those workers interested in structure-activity relationships. It was considered of interest, therefore, to compare the relative activities of the major anabolic steroids by the same procedure. Data for twelve androgenic-anabolic steroids and testosterone are presented in this report. Data on the activities of these same compounds in intact rats has been reported [7].

MATERIALS AND METHODS

All rats used in these studies were obtained from the Holtzman Company, Madison, Wisconsin. Animals were 24–25 days old when castrated and were started on steroid injections one day following surgery. Six to eight rats were employed in each treatment group. All animals were maintained in temperature-controlled, air-conditioned quarters and were permitted food and water *ad libitum*. Steroids were administered subcutaneously for seven consecutive days with autopsy on the day after the last treatment day. Most of the preparations used in this study are administered orally when used clinically. However, since some of the steroids studied are either inactive or only weakly effective by the oral route (e.g. testosterone, chlorotesterone acetate, and nandrolone phenpropionate), while all are effective subcutaneously in rats, the latter route of administration was employed for all compounds. All steroids were injected in a volume of 0·25 ml/day as suspensions in a vehicle which consisted of 0·9% sodium chloride, 0·9% benzyl alcohol, 0·5% carboxymethylcellulose and 0·4% Polysorbate 80 in distilled water.

The compounds studied were:

Oxymetholone (Adroyd)
17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one.

Oxandrolone (Anavar)
17β-hydroxy-17α-methyl-2-oxa-5α-androstan-3-one.

Methandrostenolone (Dianabol)
17β-hydroxy-17α-methyl-1,4-androstadien-3-one.

Nandrolone phenpropionate (Durabolin)
17β-hydroxy-4-estren-3-one, 17-phenylpropionate.

Norbolethone (Genabol)
± 13β, 17α-diethyl-17β-hydroxy-4-gonen-3-one.

Ethylestrenol (Maxibolin)
17α-ethyl-17β-hydroxy-4-estrene.

Bolasterone (Myagen)
7α-17α-dimethyl-17β-hydroxy-4-androsten-3-one.

Norethandrolone (Nilevar)
17α-ethyl-17β-hydroxy-4-estren-3-one.

Oxymesterone (Oranabol)
4,17β-dihydroxy-17α-methyl-4-androsten-3-one.

Methenolone acetate (Primobolan)
17β-hydroxy-1-methyl-5α-1-androsten-3-one, 17-acetate.

4-Chlorotestosterone acetate (Steranabol)
4-chloro-17β-hydroxy-4-androsten-3-one, 17-acetate.

Testosterone
17β-hydroxy-4-androsten-3-one.

Stanozolol (Winstrol)
17β-hydroxy-17α-methyl-5α-androstano[3,2-*c*] pyrazole.

RESULTS AND DISCUSSION

All of the steroids tested increased the weights of the seminal vesicles (Table 1), ventral prostate (Table 2), and the levator ani muscle (Table 3). The effects on all three parameters were dose-dependent and all compounds manifested linear log dose-response relationships on all three target organs. The regression data shown in Table 4 indicate that the slopes (*b*) of dose-response lines varied considerably. Slopes for the seminal vesicle response varied from 38·4 to 262·6, while the range for the ventral prostate response (23·3 to 132·9) was about half of that for the seminal vesicles. The range of slope values for the levator ani response (11·5 to 32·6) was the least of the three target organ parameters. Several of the dose response curves for the levator ani approached parallelism. Testosterone had the steepest slope on all three target organs. With any individual compound the slope of the line for seminal vesicle response was greater than that for the ventral prostate effect, which, in turn, was greater than that for the levator

Table 1. Effects of anabolic steroids on seminal vesicle weight in castrated rats

Compound	Mean seminal vesicle weight (mg/100 g) ± standard error				
	μg/rat/day				
	10	30	100	300	1000
Oxymetholone		15·3 ± 0·4	16·9 ± 0·7	33·2 ± 1·8	76·5 ± 1·5
Oxandrolone		15·4 ± 1·0	20·0 ± 1·1	43·3 ± 1·3	89·5 ± 4·4
Methandrostenolone		15·1 ± 0·6	15·2 ± 0·9	21·4 ± 2·0	59·7 ± 5·7
Nandrolone phenpropionate	15·3 ± 0·7	21·5 ± 0·7	56·0 ± 3·7	134·3 ± 5·1	171·2 ± 9·9
Norbolethone	14·7 ± 0·6	18·5 ± 1·2	41·7 ± 1·7	89·6 ± 2·3	112·1 ± 7·0
Ethylestrenol		16·7 ± 0·5	19·0 ± 1·7	35·1 ± 3·1	55·2 ± 3·2
Bolasterone	17·1 ± 1·9	29·5 ± 2·2	68·8 ± 4·3	159·8 ± 9·6	206·8 ± 6·4
Norethandrolone	14·2 ± 1·3	15·8 ± 0·8	21·5 ± 0·8	65·5 ± 2·6	113·0 ± 6·0
Oxymesterone	15·4 ± 1·0	15·7 ± 1·1	28·8 ± 2·7	96·0 ± 3·4	136·0 ± 4·7
Methenolone acetate	16·4 ± 0·6	23·7 ± 2·1	50·8 ± 5·4	111·5 ± 3·9	146·3 ± 4·9
Chlorotestosterone acetate	16·0 ± 0·5	16·3 ± 0·7	20·1 ± 1·0	44·0 ± 2·8	83·9 ± 4·8
Stanozolol	14·8 ± 0·5	19·7 ± 0·7	25·9 ± 0·9	64·9 ± 6·3	87·5 ± 6·6
Testosterone	19·8 ± 0·6	34·0 ± 3·5	104·9 ± 11·1	230·4 ± 6·1	250·6 ± 9·0
Composite controls (122 rats) = 15·3 ± 0·2					

Table 2. Effects of anabolic steroids on ventral prostate weight in castrated rats

Compound	Mean ventral prostate weight (mg/100 g) ± standard error				
	μg/rat/day				
	10	30	100	300	1000
Oxymetholone		13·6 ± 1·3	18·9 ± 0·9	37·6 ± 2·3	73·9 ± 4·9
Oxandrolone		13·8 ± 0·6	20·1 ± 2·3	39·3 ± 2·3	69·4 ± 4·7
Methandrostenolone		11·1 ± 0·5	12·0 ± 0·7	19·9 ± 2·1	49·6 ± 5·1
Nandrolone phenpropionate	11·4 ± 0·7	18·4 ± 2·1	39·1 ± 3·0	91·4 ± 5·2	121·6 ± 5·2
Norbolethone	12·8 ± 0·6	18·2 ± 1·6	36·1 ± 5·4	61·9 ± 4·7	85·9 ± 6·6
Ethylestrenol		13·3 ± 1·0	18·9 ± 2·1	26·6 ± 3·0	42·1 ± 2·5
Bolasterone	18·2 ± 2·4	38·4 ± 1·0	55·6 ± 3·3	121·6 ± 8·2	154·1 ± 10·3
Norethandrolone	10·9 ± 0·3	15·6 ± 1·0	19·7 ± 1·3	43·2 ± 3·0	85·8 ± 11·7
Oxymesterone	13·4 ± 0·5	15·9 ± 1·2	27·5 ± 3·3	74·9 ± 5·0	115·2 ± 2·6
Methenolone acetate	12·1 ± 0·5	24·5 ± 3·4	38·6 ± 3·3	74·4 ± 5·5	97·4 ± 2·9
Chlorotestosterone acetate	11·7 ± 0·5	16·0 ± 1·2	18·4 ± 2·7	38·4 ± 2·7	57·4 ± 4·1
Stanozolol	10·8 ± 0·6	17·1 ± 1·2	28·3 ± 1·5	48·3 ± 1·6	61·6 ± 3·3
Testosterone	16·3 ± 1·8	26·8 ± 2·4	77·9 ± 8·0	160·4 ± 9·2	161·4 ± 6·9
Composite controls (122 rats) = 11·9 ± 0·2					

ani weight. This would indicate that within the linear portion of their respective dose-response curves a given increment in dose results in weight increases for seminal vesicles > ventral prostate > levator ani.

The differences in slopes of the dose response curves are probably reflections of variations in absorption, binding, distribution, metabolism and excretion patterns of the various steroids employed, as well as the receptor affinity and relative androgenic and myotrophic activities of the compounds. Little is known about the relative pharmacodynamic properties of anabolic steroids, and the use of a seven day treatment period may not be optimal for the determination of activity,

Table 3. Effects of anabolic steroids on levator ani weight in castrated rats

Compound	Mean levator ani weight (mg/100 g) ± standard error μg/rat/day				
	10	30	100	300	1000
Oxymetholone		23·0 ± 1·3	30·9 ± 1·8	38·4 ± 2·6	48·9 ± 4·3
Oxandrolone		25·3 ± 1·7	33·6 ± 1·3	47·2 ± 1·2	54·8 ± 3·9
Methandrostenolone		23·9 ± 1·2	28·2 ± 0·9	33·5 ± 1·6	44·5 ± 2·2
Nandrolone phenpropionate	30·1 ± 0·9	46·5 ± 2·1	58·0 ± 4·4	65·7 ± 1·9	66·5 ± 2·6
Norbolethone	27·2 ± 1·0	43·1 ± 2·0	52·2 ± 2·4	60·6 ± 2·2	59·4 ± 2·6
Ethylestrenol		25·4 ± 1·1	38·5 ± 2·0	45·5 ± 1·8	58·7 ± 4·3
Bolasterone	28·1 ± 1·5	39·5 ± 2·0	54·2 ± 2·3	62·7 ± 1·9	68·2 ± 3·9
Norethandrolone	23·0 ± 0·8	31·0 ± 1·3	48·6 ± 2·5	53·9 ± 3·3	61·9 ± 3·4
Oxymesterone	24·5 ± 1·4	29·1 ± 1·3	43·5 ± 1·6	53·7 ± 3·2	62·2 ± 2·9
Methenolone acetate	26·2 ± 1·5	38·7 ± 2·7	44·0 ± 2·3	53·7 ± 1·7	61·1 ± 2·5
Chlorotestosterone acetate	21·5 ± 1·0	27·2 ± 1·7	37·4 ± 1·9	47·9 ± 2·5	53·6 ± 1·8
Stanozolol	24·0 ± 1·3	34·9 ± 1·9	39·4 ± 1·5	45·4 ± 1·5	52·3 ± 2·2
Testosterone	26·8 ± 1·2	29·4 ± 1·4	44·6 ± 3·2	62·1 ± 2·1	71·4 ± 2·6
Composite control (122 rats) = 22·6 ± 0·3					

Table 4. Regression data*

Compound	Seminal vesicles		Ventral prostate		Levator ani	
	b	*a*	*b*	*a*	*b*	*a*
Oxymetholone	82·6	−171·4	55·2	−94·1	18·0	−5·5
Oxandrolone	88·2	−175·0	49·4	−80·2	20·2	−4·9
Methandrostenolone	73·1	−159·6	56·7	−120·4	16·4	−5·4
Nandrolone phenpropionate	163·8	−271·3	82·0	−120·4	23·9	8·6
Norbolethone	100·2	−158·5	49·6	−62·6	22·0	7·5
Ethylestrenol	38·4	−59·8	23·3	−28·8	20·4	−3·7
Bolasterone	190·4	−311·6	81·5	−90·0	23·9	4·5
Norethandrolone	90·7	−158·9	66·3	−115·7	19·6	5·0
Oxymesterone	140·6	−252·2	87·4	−145·5	21·7	−1·4
Methenolone acetate	127·1	−203·2	50·2	−53·8	15·2	15·4
Chlorotestosterone acetate	76·2	−144·5	38·9	−59·0	20·7	−3·5
Stanozolol	81·6	−137·2	33·1	−36·5	11·5	17·2
Testosterone	262·6	−419·7	132·9	−175·1	32·6	−19·3

*$y = b \log x + a$, where x = dose in μg and y = organ weight (mg/100 g).

Regression equations were calculated by the method of least squares using 2 dose-response points for seminal vesicles and 3 to 4 points for ventral prostate and levator ani. Points were selected which appeared to be on the steep, linear portion of the dose-response curves as determined by visual inspection of the graphed, experimental data.

especially with compounds which differ in their duration of action, e.g. nandrolone phenpropionate, a long-acting steroid. It is also possible that each organ may differ in the time required for maximal response to a given dose of compound. Hence, potency rank could vary significantly when different treatment schedules are used. In the present study the seven day treatment period of Hershberger *et al.*[8] was employed.

With non-parallel dose-response lines exact estimates of relative potency are not possible. Comparison of the required dosages of two compounds at a given

level of response results in a different ratio of potency at the convergent segment of the dose-response lines than it does at the divergent portion. The same situation exists with non-parallel curves for two parameters, as in the computation of the "androgenic/anabolic ratio". It is obvious that favorable or unfavorable ratios can be obtained for a given compound depending on the selection of suitable response levels for comparison of dosages and subsequent ratio calculation.

The selection of a given response level to be used for comparisons of potency, when non-parallel dose response curves are involved, is purely arbitrary. It was decided in the present study to evaluate potencies in terms of the dosages required to double the weights of the respective target organs. The doubling dose was selected because all of the compounds produced at least a minimum two fold increase in the least responsive parameter, levator ani weight, at the highest dosage tested. Selection of a higher response level for comparison of myotrophic potencies would have necessitated extrapolation and assumption of linearity beyond the observed experimental dose-response range. Levator ani responses of less than 100% increase above controls are occasionally not statistically different from controls and are in the region where dose response curves converge with consequent similar compound potencies. Since it was desirable to compare all three parameters at the same response level, seminal vesicle and ventral prostate activities were also compared in terms of doubling doses. Doubling doses for the evaluation of anabolic potency have been employed by Falconi[9], and

Table 5. Potency rank according to calculated doubling dose (DD)

Seminal vesicles	DD (μg/day)	Ventral prostate	DD (μg/day)	Levator ani	DD (μg/day)
Testosterone	52	Bolasterone	25	Nandrolone†	34
Bolasterone	63	Testosterone	31	Bolasterone	50
Methenolone*	69	Methenolone*	35	Norbolethone	52
Nandrolone†	70	Norbolethone	55	Methenolone*	91
Norbolethone	77	Nandrolone†	57	Testosterone	95
Oxymesterone	103	Stanozolol	66	Norethandrolone	113
Stanozolol	114	Oxymesterone	87	Oxymesterone	140
Norethandrolone	123	Norethandrolone	127	Chlorotestosterone*	225
Chlorotestosterone*	199	Oxandrolone	127	Ethylestrenol	250
Oxandrolone	214	Chlorotestosterone*	134	Stanozolol	272
Ethylestrenol	226	Oxymetholone	137	Oxandrolone	302
Oxymetholone	279	Ethylestrenol	181	Oxymetholone	656
Methandrostenolone	400	Methandrostenolone	349	Methandrostenolone	1217

*Acetate.
†Phenpropionate.

Edgren[1] has reported doubling doses for levator ani and ventral prostate weights.

The doses required to increase organ weights 100% above the composite control weights were calculated from the regression equations for each compound for each of the three parameters. The "doubling doses" are listed in Table 5 and tabulated in order of potency. It can be seen in Table 5 that although the doses required to double the weights of the seminal vesicles and the ventral prostate differ significantly for an individual compound, the potency rank within the series of steroids is very similar for both target organs. In all cases the potency

rank measured by seminal vesicle activity is within one position of that measured by ventral prostate response. This similarity of compound sequence would indicate that these two organs react in the same way to various modifications of the steroid structure. When doubling doses for levator ani weight were tabulated in order of potency, a different sequence of compounds was obtained. This would suggest that the structural requirements for levator ani activity differ from those for the seminal vesicles and ventral prostate, and indicate that it may be possible by molecular manipulation to obtain compounds with selective effects on either the androgenic parameters (seminal vesicles and/or ventral prostate) or the anabolic parameter (levator ani).

ACKNOWLEDGEMENTS

We are grateful to the following pharmaceutical firms for supplies of steroids: Ciba (Dianabol), Farmitalia (Steranabol and Oranabol), Organon (Durabolin and Maxibolin), Parke-Davis (Adroyd), Schering AG (Primobolan), Searle (Anavar and Nilevar), Upjohn (Myagen), Winthrop (Winstrol) and Wyeth (Genabol).

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