SPECIAL LECTURE**Evaluation of Anabolic Steroids*****G.A. OVERBEEK, J. VAN DER VIES, J. DE VISSER**

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Research preceding clinical use of drugs aims at the best possible prediction of the effects in man.

The Chemist envisaging new structures has only very few leads that will enable him to prepare a drug with the desired properties. If the drug should be an anabolic steroid these properties should be high anabolic activity, absence of androgenicity and other side effects. In order to achieve this he will prepare derivatives either of androstan-17 β -ol or 19-nor-androstan-17 β -ol. If he wants an orally active substance he will introduce a 17 α -methyl- or ethyl group. If he wants a long acting parenteral drug he will esterify the 17 β -hydroxy group. Other changes that have been or are likely to be successfully applied, such as double bonds or substitutions at the carbon atoms 1, 2, 4, 6, 7, 9, 11, 16 or 18 are mainly haphazard.

The animal pharmacologist is in a better position. A wealth of data obtained by the study of many compounds with only a few methods are available and at least a number of the compounds have also been applied in man. This would make it fairly easy to judge the value of the methods, were it not that the final results obtained with one and the same approach e.g. the levator ani method may differ greatly depending on choice of criterion, rate and frequency of administration, and mathematical processing of the experimental data (1).

The latter should if possible lead to one or two figures that make it easy for the clinician to select the preparation most suitable for the particular patient. Preferable is the use of potency ratio's ($R_{anab.}$ and $R_{andr.}$) expressing the anabolic and androgenic activity in terms of the activity of a standard preparation.

$\left(R = \frac{\text{activity "unknown"}}{\text{activity standard}}\right)$. From these potency ratio's an anabolic/androgenic ratio $Q = \frac{R_{anab.}}{R_{andr.}}$ can be calculated.

(It should be remarked that other investigators use the term anabolic/androgenic ratio and the same letter Q in a quite different way which makes these terms not comparable to those mentioned above.)

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Legend to Fig. 1. Upper part : Calculated log dose response regression lines for levator ani weights.
Lower part : The same for log seminal vesicle weights.

- ▲ — ▲ ethynandrol
- — ○ norethandrolone
- — ● methyltestosterone

These R and Q values are calculated from the dose-response curves. They are independent of the dose in the dose range where these curves are parallel. Other calculations making use of only one particular point of the curve, such as the threshold dose or the dose required for substitution to normal, involve the fact that when the dose response curves are not parallel the results apply only to the dose level studied, of which no one can judge the equivalent in the human.

Although strict parallelism of dose response curves of structurally different compounds probably does not exist, the curves were in a number of cases found not to deviate statistically significantly from parallelism. Thus it is admissible to calculate regression lines for the various substances having an average common slope as shown in figure 1 for the anabolic and androgenic activities of ethylnandrol and norethandrolone compared with methyltestosterone as a reference preparation. Table 1 shows the values for $R_{anab.}$ and $R_{andr.}$ and Q calculated from these data. The Q value for ethylnandrol^{x)} is one of the highest we found, when studying a great number of various steroids (2).

The use of different criteria leads to different results. The measurements of nitrogen retention (3) gives quite different values for anabolic activity from that based

Table 1. Potency ratio's (R_{MLA} and $R_{sem. ves.}$) and anabolic/androgenic ratio's (Q) of ethylnandrol and norethandrolone, compared with methyltestosterone.

	norethandrolone/ methyltestosterone	ethylnandrol/ methyltestosterone	ethylnandrol/ norethandrolone
R_{MLA}	1.0	4.2 ^{x)}	4.1 ^{x)}
$R_{sem. ves.}$	0.34 ^{x)}	0.22 ^{x)}	0.66 ^{x)}
$Q = \frac{R_{MLA}}{R_{sem. ves.}}$	3.1	19.0 ^{x)}	6.2 ^{x)}

x) Statistically significantly different from 1.

Table 2. N-Retention and Levator and in the rat as criteria for anabolic activity Potency ratio's ($R_{anab.}$) compared with methyltestosterone.

Compound	$R_{anab.}$	
	N-ret	M.L.A.
stanazolol	10.0 (5)	2.0 (6) 2.6
ethylnandrol	1.7 (5)	4.2 6.7 6.4 5.0 3.1

5.1

x) Orgabolin, Organon.

Table 3. Seminal vesicles and ventral prostate in the rat as criteria for androgenic activity.
Potency ratio's ($R_{andr.}$) compared with methyltestosterone.

Compound	$R_{andr.}$	
	sem. ves.	ventr. prost.
ethylnandrol	0.34	0.073
	0.22	0.15
	0.52	0.085
		0.22
2-carboxy-17 α -methyl-	0.18	0.48
5 α -androst-2-en-17 β -ol	(0.06-0.28)	(0.40-0.57)
3-methylene-17 α -methyl-	0.84	0.32
5 α -androstan-17 β -ol	(0.52-1.18)	(0.25-0.41)

on the myotrophic effects as determined by the levator ani method (4). Great differences are also found when evaluating the androgenic activity by means of growth either of seminal vesicles or ventral prostate. The use of a standard preparation does not eliminate these difficulties since different steroids affect both organs in a different, unrelated and hence unpredictable manner. Some particularly striking examples of such differences both with respect to anabolic and androgenic activity are presented in tables 2 and 3. It is obviously impossible to decide from these values which ones are correct and hence which of the criteria can be used best for assaying.

The assay method to be selected must meet the following two requirements:

1. The highest possible accuracy should be obtained.
2. There should be the best possible agreement between animal and clinical data.

With respect to the first one the levator ani assay has undoubtedly many advantages over other tests such as the nitrogen retention test in the rat. Since it is simple and not at all laborious, one assay can easily be done with a rather large number of rats, and the dissecting and weighing of the levator ani muscle, seminal vesicles and ventral prostate can be done with high precision by skilled but not necessarily highly educated personnel. It is always much more difficult to prove for any bioassay if the second requirement is met, and this is very much the case with respect to anabolic activity, applied therapeutically for many different purposes where the criteria are different and the effects are difficult to express in a figure. In order to have the best chance of meeting the second requirement, the conditions of the bioassay should be made as comparable as possible to those existing during therapy. Because of the difference in wanted effects in different therapeutic indications it is more or less hopeless to aim at the use of the same criterium for the bioassay. However there are two points of particular importance that should be stressed and fortunately can be realized e.g. with respect to route and to frequency of administration. Oral preparations have to be administered by the oral route and parenteral long acting preparations should be injected

only once, or weekly or biweekly and not daily as is often done. The influence of different patterns of administration are demonstrated by the results of the following experiments with nandrolone, nandrolone phenylpropionate^{x)} and nandrolone decanoate^{xx)} (8).

In the course of studies aiming at finding whether nandrolone esters act as such on the end organs or rather after previous hydrolysis to nandrolone, we compared the effect of the esters with that of free nandrolone administered following three different schedules of injection.

Table 4 demonstrates the very marked differences found in the effects of nandrolone when injected in different dosage schedules on the levator ani muscle and the seminal vesicle of castrated rats in the Hershberger test. One single injection had hardly any effect. The same total dose divided over 21 equal sub doses administered three times daily for one week led to marked growth of both levator ani and seminal vesicles. After administration of the esters in doses causing comparable effects on the levator ani

Table 4. Influence of the mode of administration on the effects of nandrolone

Substance	total dose (mg)	mode of administration	Increase in weight in mg			
			after 1 week		after 2 weeks	
			M.L.A.	Sem. Vem.	M.L.A.	Sem. Ves.
nandrolone	1.75	single dose	3.3	1.5	5.3	0.5
nandrolone phenylpropionate	1.00 ¹⁾	single dose	33.0	16.6		
nandrolone	1.75	simulation	30.0	17.2		
nandrolone	1.75	21 equal doses	33.8	33.6		
nandrolone						
decanoate	1.00 ¹⁾	single dose	25.0	8.8	34.5	6.3
nandrolone	1.75	simulation	25.5	6.9	31.0	5.0

1) these doses were selected in order to obtain comparable effects on the M.L.A.

Table 5. Dosis schedule used for the "simulation" of the effects of the ester as recorded in table 4. Percentage of total dose of nandrolone administered every 8th hour.

a. for simulating the action of nandrolone phenylpropionate

day	1	2	3	4	5	6	7
20	8	8	6	3	2	1	$\frac{1}{2}$
16	8	8	5	2	1	1	$\frac{1}{2}$
12	7	7	3	2	1	$\frac{1}{2}$	$\frac{1}{2}$

b. for simulating the action of nandrolone decanoate

day	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4	4	3	3	$2\frac{1}{2}$	2	2	$1\frac{1}{2}$	2	$1\frac{1}{2}$	1	1	1	$\frac{1}{2}$	
4	4	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	2	$1\frac{1}{2}$	$1\frac{1}{2}$	1	1	1	1	$\frac{1}{2}$	
4	4	$2\frac{1}{2}$	$2\frac{1}{2}$	$2\frac{1}{2}$	2	1	$1\frac{1}{2}$	$1\frac{1}{2}$	$1\frac{1}{2}$	1	1	1	1	

x) Durabolin Organon.

xx) Deca-Durabolin, Organon.

the effects on the seminal vesicles were less marked. When nandrolone was administered again in subdivided doses, but this time not equal but calculated from the chemically determined disappearance rates of the esters from the site of injection, effects comparable to those of the respective esters were found. The calculated "simulating" doses schedule used is given in table 5.

These data are not only of interest in demonstrating the marked influence of the dosage schedule on the effect of nandrolone, but they also show that too frequent f.i. daily injection of the esters must necessarily lead to a different anabolic/androgenic ratio of these esters. In this case amounts of nandrolone reaching the end organs per unit of time, determining the effect will be closer to the "equal" subdivision than to the "simulating" subdivision of the dose.

The human pharmacologist is able to obtain an impression of the anabolic activity of steroids by studying their effects on the nitrogen balance in normal subjects. Because of the labour and time involved he will rarely be able to make dose-response curves and he will usually compare the substances by determining their respective threshold doses. It is fortunate that these threshold doses for shifting the N-balance in a positive direction appear to be of the same order of magnitude as the average clinical dose, since for the latter purpose threshold doses are obviously uninteresting. Various anabolic steroids are being studied^{x)} but insufficient data are available as yet to allow a quantitative evaluation that could be compared with that obtained from animal assays.

The quantitative evaluation of androgenic activity in the human, especially in women and children, these being the most important subjects with respect to clinical side effects, is impossible for technical and ethical reasons.

The clinician can not provide any quantitative information on $R_{\text{anab.}}$, $R_{\text{andr.}}$ and Q . This is very unfortunate since quantitative data on clinical activity would obviously be of still greater practical importance than the human pharmacological data.

Table 6. Anabolic potency ratio's (R_{MLA} and $R_{\text{N-ret.}}$) and recommended clinical dose (in U.S.A.)

Compound	I R_{MLA}	II $R_{\text{N-ret.}}$	III mean clinical dose (mg)	ranking order		
				I	II	III
ethylnandrol	5.1	1.7	2-4	1	4	1
stanazolol	2.6	10.0	6	2	1	3
norethandrolone	1.0	3.9	20-30	3	2	5
methandrostenolone	0.9	1.2	2.5-5	4½	5	2
oxymetholone	0.9	1.75	5-10	4½	3	4

Reference standard for R_{MLA} and $R_{\text{N-ret.}}$: methyltestosterone.

Rank correlation coefficients (Spearman):

I - III +0.42

II - III -0.40

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It was thought that perhaps some impression might be gained by comparing the doses recommended by the pharmaceutical houses for their own preparations, though it was realized that factors other than anabolic activity influence this choice of dose. Hence the evidence obtained from the comparison can at best be considered as circumstantial. The doses are those recommended in the U.S.A. The data recorded in table 6 show that the mean clinical doses for ethylndrol, stanazolol and methandrostenolone are of the same order of magnitude, whereas oxymetholone and norethandrolone are less active. This correlates better with the results of the levator ani-test than with those of the N-retention test in the rat as can be concluded from the rank correlation coefficients according to Spearman (9) (table 6).

SUMMARY

Research preceding clinical use of drugs aims at the best possible prediction of the effects in man. In the case of anabolic steroids the predictive value of the levator ani assay appears to be satisfactory, and the precision of the figures obtained to be high. Experimental data are presented demonstrating that the results found in the same test with the same substances may be very different depending on the criterium used and the route and frequency of administration.

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