ANABOLIC PROPERTIES OF ETHYLESTRENOL

By

G. A. Overbeek, A. Delver and J. de Visser

ABSTRACT

The anabolic and androgenic properties of ethylestrenol, norethandrolone and methyltestosterone were compared by means of a quantitative levator ani assay (Hershberger 1953). In a second assay of this kind ethylestrenol and methandrostenolone were compared. In both instances ethylestrenol was shown to be a potent anabolic substance with by far the highest anabolic/androgenic ratio.

de Winter et al. (1959) described a new series of steroids, the 17-alkylated 17β-hydroxy-estr-4-enes (or estrenoles). The present paper deals with the 17α-ethyl-derivative which proved to be a potent anabolic substance with a very low androgenic activity. The compound was mainly studied by the levator ani assay (Hershberger et al. 1953), following the principles of comparison described in the paper by Overbeek & de Visser (1961). The effect of ethylestrenol was compared with that of some other orally active anabolic substances, i.e. methyltestosterone, norethandrolone (17α-ethyl-19-nortestosterone = 17α-ethyl-17β-hydroxy-19-norandrost-4-en-3-one) (Saunders & Drill 1956) and methandrostenolone (Δ¹-methyl testosterone = 17β-hydroxy-17α-methylandrosta-1,4-dien-3-one) (Desaulles et al. 1959).

METHODS

For experiment I, rats of about 50 g bodyweight were castrated and the substances were administered orally as microcrystalline suspensions once daily for 7 days, be-

1. Ethylestrenol is the generic name of 17α-ethyl-17β-hydroxy-estr-4-ene. It is the active principle of the preparation Orgabolin (Organon).
ginning on the day after castration. Six rats were used for each dose level (1/4, 1/2, 1, 2, 4 and 8 mg per day per rat) of each of the three substances.

In addition, three groups of six rats were used as controls, so that the whole experiment comprised 126 rats. As all the autopsies could not be done on one day, they were divided into three equal blocks, treated and autopsied with phase-differences of one day from block to block. Each block was so arranged as to comprise one third of each treatment group, thus ensuring independence of treatment and possible block effects.

In experiment II eight rats were used for each of the doses 1/8, 1/4, 1/2, 1, 2 and 4 mg ethylestrenol and 1/4, 1/2, 1, 2, 4 and 8 mg of methandrostenolone. Here two blocks were used with a phase-difference of one day.

**RESULTS**

*Experiment I.* — A comparison was made between the effects of ethylestrenol, norethandrolone and methyltestosterone, the latter substance serving as a reference preparation.

As the distributions of the levator ani weights within treated groups are reasonably normal with approximately constant scatter, no transformation was necessary. Mean levator ani weights plotted against log dose yielded regression lines as shown in Fig. 1.

Only the highest three mean values had to be discarded owing to a bending of the curves to a maximum level. All the values for the low doses could be used.

The analysis of variance showed the following results:

a. Difference between treatments are reproduced from block to block.

b. No significant deviation from linearity occurs in any of the three response curves.

c. No significant deviations appear between the three linear regression coefficients.

d. There is a highly significant common linear regression.

The efficiency of this response is only moderate: $\lambda$ (the standard deviation per observation divided by the regression coefficient) = 0.33.

Taking the anabolic activity of methyltestosterone as equal to 1, the anabolic activity of norethandrolone is 1.0 (0.7–1.5) and that of ethylestrenol 4.2 (2.8–6.6).

The anabolic activity of ethylestrenol with regard to norethandrolone is 4.1 (2.6–6.6). The confidence of all the intervals shown in this paper is 95%.

The curve of distribution of seminal vesicle weights within groups is very skew with the long tail in the direction of high values. Moreover, the scatter increases approximately in proportion to the mean value. Therefore, a logarith-
Fig. 1.

Experiment I (see methods).
Upper part: Calculated log dose - response regression lines for levator ani weights.
Lower part: The same for log seminal vesicle weights.

A—ethylestrenol
O—norethandrolone
●—methyltestosterone

mic transformation was applied, making the distribution approximately normal, at the same time equalizing the scatter.

Obviously the appearance of the regression lines is also altered by the transformation. The three new curves are presented in the lower part of Fig. 1.

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Fortunately, as a third effect of the transformation, the curves are reasonably linear, if at least the lowest doses of the two least active compounds, which hardly show any effect, are omitted. The analysis of variance of the remaining observations shows that:

a. Differences between treatments are reproduced from block to block, though significant differences between blocks occur.
b. No significant deviation occurs from linearity in any of the three dose-response curves.
c. No significant deviations appear between the three linear regression coefficients.
d. There is a highly significant common linear regression.

Thus, once again all the conditions, necessary for the evaluation of valid potency ratios, are satisfied. The efficiency is better than in the m. l. a.-test: \( \lambda = 0.22 \). Taking the androgenic activity of methyltestosterone as 1, the androgenic potency of norethandrolone is 0.34 (0.26–0.43) and that of ethylestrenol 0.22 (0.17–0.29).

The androgenic potency of ethylestrenol with regard to norethandrolone is 0.66 (0.51–0.85).

Of special interest is the anabolic activity as compared with the androgenic activity. The calculation of potency ratios as applied above allows the introduction of an anabolic/androgenic ratio.

We therefore define the anabolic/androgenic potency ratio \( Q_{(T, S)} \) between the »test substance« T and the »standard« S, as

\[
Q_{(T, S)} = \frac{R_{\text{ana}}(T, S)}{R_{\text{and}}(T, S)}
\]

where \( R_{\text{ana}}(T, S) = \) anabolic potency ratio between T and S

\( R_{\text{and}}(T, S) = \) androgenic

For instance, if T is three times as anabolic and twice as androgenic as S, \( Q_{(T, S)} = \frac{3}{2} = 1.5 \). In general, since in the case of anabolic steroids high anabolic and low androgenic activity are desired, we might say that T is \( Q_{(T, S)} \) times »good« as S.

In this way any two compounds of the three investigated can be compared, starting from the ratio of anabolic potencies and the ratio of androgenic potencies, and these ratios are once more presented below:

<table>
<thead>
<tr>
<th></th>
<th>methyltestosterone</th>
<th>norethandrolone</th>
<th>ethylestrenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anabolic potency ratio</td>
<td>1</td>
<td>1.028</td>
<td>4.207</td>
</tr>
<tr>
<td>Androgenic</td>
<td></td>
<td>1.028</td>
<td>4.207</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.335</td>
<td>0.222</td>
</tr>
</tbody>
</table>

From the above figures we derive the three anabolic/androgenic potency ratios:
\[
Q_{(\text{norethandrolone, methyltestosterone})} = \frac{1.028}{0.335} = 3.1
\]
\[
Q_{(\text{ethylestrenol, methyltestosterone})} = \frac{4.207}{0.222} = 19.0
\]
\[
Q_{(\text{ethylestrenol, norethandrolone})} = \frac{4.208 : 1.028}{0.222 : 0.335} = 6.2
\]

Each Q-value is a ratio of two potency ratios. The fact that these two are not independent (since they are based on the same sample of rats), makes it impossible to compute the accuracy of Q from the accuracy of each of the two potency ratios.

Hence, to obtain an impression of the confidence to be put in the Q-values, the whole computation has been repeated separately for each of the three blocks which constitute the whole experiment. The Q-values thus calculated will be independent from block to block and will thus reflect the reproducibility of the Q-values per block. The results are presented in Table 1.

For each of the three kinds of Q deviation from unity can be tested. It appears that under the conditions of this experiment only Q \((\text{norethandrolone, methyltestosterone})\) does not differ significantly from unity.

**Experiment II.** – The comparison of ethylestrenol with methandrost enolone yielded the following results (see Fig. 2).

Putting the anabolic activity \(R_{\text{ana}}\) of methandrost enolone as equal to 1, \(R_{\text{ana}}\) of ethylestrenol appeared to be: 13.1 \((7.2-28.3)\).

The androgenic potency ratio \(R_{\text{and}}\) calculated on the basis of:

\[
\text{seminal vesicle weight} = 1.46 \ (0.97-2.04)
\]
\[
\text{ventral prostate weight} = 0.93 \ (0.47-1.65)
\]

<table>
<thead>
<tr>
<th>(Q_{(\text{norethandrolone, methyltestosterone})})</th>
<th>(Q_{(\text{ethylestrenol, methyltestosterone})})</th>
<th>(Q_{(\text{ethylestrenol, norethandrolone})})</th>
</tr>
</thead>
<tbody>
<tr>
<td>block 1</td>
<td>2.3</td>
<td>13.5</td>
</tr>
<tr>
<td>block 2</td>
<td>6.2</td>
<td>32.1</td>
</tr>
<tr>
<td>block 3</td>
<td>1.9</td>
<td>18.0</td>
</tr>
<tr>
<td>Total experiment</td>
<td>3.1</td>
<td>19.0</td>
</tr>
</tbody>
</table>
Fig. 2.
Experiment II (see methods).
Upper part: Calculated log dose - response regression lines for levator ani weights.
Lower part: The same for log seminal vesicle weights.

Apart from possibly different androgenic effects on the target organs seminal vesicle and ventral prostate, it appeared from the limits of confidence that the $R_{\text{and}}$ calculated on the basis of the seminal vesicle weight was considerably more accurate.

$$ Q = \frac{R_{\text{ana}}}{R_{\text{and}}} $$

of ethylestrenol/methandrostenolone was:

$Q_s = 8.9$ (responses: levator ani and seminal vesicle weights).

**DISCUSSION**

The above results demonstrate the high myotrophic and low androgenic properties of ethylestrenol. In both experiments the anabolic/androgenic ratio ($Q$)
is high, whether compared with methyltestosterone, norethandrolone or methandrostenolone.

It should be realized that the Q-values are ratios which do not yield information about absolute or even relative potency. The latter can be read from the R-values.

No essential difficulties such as non-parallelism of dose-response curves occurred which would have prevented a reliable comparison. Apparently the substances used in this study are sufficiently similar in this respect to be compared. They meet the requirements mentioned in the paper by Overbeek & de Visser (1961).

As anticipated on the basis of the data reported, the available clinical experience appears to demonstrate that ethylestrenol is a potent oral anabolic agent with low androgenic activities, although exactly the same relative figures do not necessarily apply.

REFERENCES


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