EFFECTS OF TESTOSTERONE AND NANDROLONE AND SOME OF THEIR ESTERS ON THE PSEUDOCHOLINESTERASE ACTIVITY IN THE LIVER AND SERUM AND ON THE SEMINAL VESICLE AND LEVATOR ANI MUSCLE OF THE RAT

By

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ABSTRACT

Testosterone (T), testosterone-propionate (TP), testosterone-phenyl-propionate (TPP), nandrolone (N) (19-nor-testosterone) and nandrolone-phenyl-propionate (NPP) were compared for their effects on the pseudocholinesterase activities in the liver and serum of castrated male rats. In addition changes in the weight of the seminal vesicle and the levator ani muscle were studied. After daily administration of 1 mg of the hormones for ten days, T and TPP showed a more marked depression of the pseudocholinesterase activity and seminal vesicle than the corresponding nor-derivatives. TP and TPP have approximately similar effects, exceeding those of T. On the levator ani N and NPP were more effective than T and TPP. At identical total doses, administration of all hormones with intervals of more than one day, produced less depression of the pseudocholinesterase activity and less seminal vesicle growth than daily administration. The effects on the levator ani were less influenced by varying intervals. At an interval of four days TPP still had a potent effect on the enzyme activity and the seminal vesicle, whereas T was almost without effect. Prolonged administration showed that the effects on the enzyme activity and the seminal vesicle of N and NPP could not reach the maximum effects of T and TPP respectively.

It has been shown previously (Leeuwin 1963, 1965, 1966) that the enzyme pseudocholinesterase is markedly influenced by the endocrine system. Adult male rats have a relatively low pseudocholinesterase activity as compared to imma-
ture male and female rats and adult females. In adult male rats, orchidectomy results in an increased enzyme activity up to the level found in immature rats. Subsequent administration of T or TP depresses the activity to the adult value. In a previous study (Leeuwin 1965) the effect of N with weaker androgenic properties and a relatively high anabolic potency (Hershberger et al. 1953; Saunders & Drill 1957; Overbeek & De Visser 1957; Edgren 1963) on the enzyme activity was compared with that of TP. At identical dose levels NPP depressed the enzyme activity of male castrates to a much smaller extent. Since it seemed more satisfactory to compare all the effects of NPP with those of TPP, a potent androgenic compound, TPP was also included in the present investigation. In order to get a more complete picture of the effects of androgenic-anabolic substances on the pseudocholinesterase activity and seminal vesicle and levator ani the effects of T and N have also been studied.

MATERIAL AND METHODS

Castrated male randomly bred rats, originating from a Wistar® strain were used in all experiments. Castration was performed on five to seven weeks old males (body weight 120-145 g). Experiments started two to three weeks after castration.

The steroids, dissolved in arachis oil, were administered by subcutaneous injection. In one series of experiments, identical total doses were injected, either in ten daily doses or in doses spaced at intervals of two (four doses), three (three doses) and four (two doses) days. In a second series the animals were injected daily for periods ranging from five to fifty days.

The animals that were injected daily were sacrificed on the day after the last injection. The animals that were injected at longer-intervals were autopsied either two, four or six days after the last injection. Serum samples and liver homogenates were prepared as described previously (Leeuwin 1965) and pseudocholinesterase activity was determined according to the Warburg technique at 37°C and expressed as mlCO₂ per gram (ml) per 20 min. Androgenic and anabolic responses were studied respectively by observing the average changes in weight of the seminal vesicles and the levator ani as compared to controls injected with oil only (Hershberger et al. 1953). An idea of the difference in effect based on the two criteria used is given by the ratio:

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\frac{\text{increase in levator ani weight}}{\text{increase in seminal vesicle weight}}
\]

RESULTS

Fig. 1 summarizes the effect of ten daily injections of T, TP, TPP, N and NPP on the pseudocholinesterase activity in the liver and serum of male castrates. All compounds except N lower the enzyme activity, even at the lowest dose level used. The effects on the activity in the liver run parallel to those on the

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Pseudo ChE activity

Liver

Serum

daily dose (µg)

daily dose (µg)

Fig. 1.
Effect of T (□), TP (○), TPP (▲), N (×) and NPP (●) on the pseudocholinesterase activity in the liver and serum of castrated male rats. The compounds were administered by subcutaneous injections during ten days. Each group consisted of 9 animals.

activity in the serum. Actually the effects at the higher dose levels can be summarized as follows: N ≪ NPP ≪ T ≲ TP ≲ TPP.

Fig. 2 shows the effect on the seminal vesicles and levator ani muscle. N and NPP have a relatively poor effect on the seminal vesicles but their effect on the levator ani is pronounced. The sequence of activity of the compounds can again be summarized as follows, on the seminal vesicle: N ≪ NPP ≪ T ≲ TP ≲ TPP and on the levator ani: T ≲ TP = TPP = N ≪ NPP; this was clearly seen at the higher dose levels. In a following series of experiments a comparison has been made between the effects of T and TPP and between the effects of N and NPP administered at varying intervals in order to examine whether there is a correlation between the effects of the esterified compounds and a »prolonged action«. The experimental design was as follows:

Exp. 1. Daily administration.
Exp. 2. Interval of two days; administration on day 1-3-7-9.
Exp. 3. Interval of three days; administration on day 1-4-7.
Exp. 4. Interval of four days; administration on day 1-5.
Fig. 2.
Effect of T (□), TP (○), TPP (▲), N (☒) and NPP (●) on the seminal vesicle and levator ani muscle weight of castrated male rats. The compounds were administrated by subcutaneous injections during ten days. Each group consisted of 9 animals.

The total doses were identical in all experiments. The result can be seen in Figs. 3, 4 and 5. In general the effects of all hormones are reduced when they are injected at longer intervals although this is less evident in the animals injected at intervals of one and two days.

Again this is particularly evident at the higher dose levels. Fig. 3 shows that at intervals of four days T is almost inactive in lowering the pseudocholinesterase activity in the liver and serum, whereas TPP, although less active than at shorter intervals, still has a relatively marked effect. To a lesser extent this is also true for the effects on the seminal vesicle; the varying intervals of administration do not appear to influence the effects on the levator ani (Fig. 4), except in the case of T given at intervals of four days. Fig. 5 shows that N is completely inactive in reducing the enzyme activity in the liver as well as in serum at any dose level and at any interval of administration. When administered at an interval of four days, NPP is also inactive. However, it can be
stated that the growth of the seminal vesicle varies with the duration of the interval. The longer the interval the smaller the effect, especially at the dose levels of 5 and 10 mg. N injected at intervals of four days has practically no effect. With some minor fluctuations, there are no indications of marked changes in the growth rate of the levator ani with the variation in the length of the interval.

Although the results summarized in Figs. 1 and 2 seem to indicate that a further increase in the dose will not cause a substantial increase in the effects of N and NPP on the pseudocholinesterase activity and the seminal vesicle weight, it cannot be excluded that prolongation of the time of administration might result in maximal effects equal to those obtained with T, TP and TPP. Experiments were, therefore, performed in which the hormones were injected during periods ranging from 0-50 days. The daily dose was 1000 µg. The results are presented in Figs. 6 and 7. Here again it is evident that it is doubtful whether NPP could ever reach the plateau obtained by TP and TPP, and at a lower level whether N will reach that of T. This applies to the pseudocholine-
Effect of T (□) and TPP (▲) on the seminal vesicle and levator ani muscle weight of castrated male rats. The compounds were administered by subcutaneous injection spaced at varying intervals. Each group consisted of 6 animals. —— interval one day —— interval two days —— interval three days . . . . interval four days.

sterase activity and to the seminal vesicle weight. The maximum effect of TP and TPP on the seminal vesicle greatly exceeds that of T, N and NPP. Differences between the effects on the levator ani are not pronounced, although the growth stimulating effect of T and N was less than that of TP and nor-TPP (Fig. 7).

The order of the MLA sem.-ves. weight ratios for the longest treatment periods is TP ≤ TPP < T < N = NPP (Table 1). In general there is a certain degree of constancy in the weight ratios within one group.

**DISCUSSION**

The potent anabolic effect and the relatively low androgenic potency of the 19-nor-derivatives of testosterone (nandrolone and its esters) is well known. In
Fig. 5.
Effect of N (×) and NPP (●) on the pseudocholinesterase activity in the liver and serum and on the seminal vesicle and levator ani muscle weight of castrated male rats. The compounds were administered by subcutaneous injection spaced at varying intervals. Each group consisted of 6 animals. —— interval one day ——— interval two days ——— interval three days . . . . interval four days.

In a previous study, it has already been shown that the effects of TP and TPP on the level of pseudocholinesterase activity of male castrates seem to correlate with their androgenic activities (Leeuwin 1965). It seemed more appropriate, however, to compare the effects of NPP with those of TPP and at the same time to include T, N and TP in the investigation.
Effect of T (□), TP (○), TPP (▲), N (×) and NPP (●) on the pseudocholinesterase activity in the liver and serum of castrated male rats. The compounds were administered by subcutaneous injections in a daily dose of 1 mg. Each group consisted of 6 animals.

In a first series of experiments ten daily injections of the hormones varying from 0.1 to 1 mg were given.

N had a smaller effect than T, and NPP a smaller effect than TPP on the pseudocholinesterase activity in the liver and serum as well as on the seminal vesicle. The effects of TP are about the same as those of TPP, especially at the higher dose levels. As to the effect on the levator ani the sequence is reversed: N and NPP have a greater effect than T and TPP respectively.

Long term treatment with the hormone confirmed the view that the maximum effect of NPP on the pseudocholinesterase activity and the seminal vesicle would never approximate the maximum effect of TPP and also that the effect of N would never approximate that of T. These observations raise a number of questions. The main questions are which processes account for these effects and what is the explanation of the fact that the non-esterified compounds have weaker actions than the (nor) PP compounds. A point to be considered is that
Table 1.
MLA - seminal vesicle weight ratios after varying treatment periods with 1 mg daily of the various substances.

<table>
<thead>
<tr>
<th>treatment time</th>
<th>T</th>
<th>TP</th>
<th>MLA/sem. ves.</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 days</td>
<td>0.55</td>
<td>0.43</td>
<td>0.59</td>
</tr>
<tr>
<td>40 days</td>
<td>0.63</td>
<td>0.52</td>
<td>0.55</td>
</tr>
<tr>
<td>50 days</td>
<td>0.69</td>
<td>0.49</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Fig. 7.
Effect of T (□), TP (○), TPP (▲), N (×) and NPP (●) on the seminal vesicle and levator ani muscle weight of castrated male rats. The compounds were administered by subcutaneous injections in a daily dose of 1 mg. Each group consisted of 6 animals.
the more marked effect of the esterified compounds might be correlated to the »prolonged action«. Therefore an experiment was performed in which T was compared with TPP and N with NPP on an injection schedule, with varying injection intervals. The result of this experiment led to a number of observations.

1. With all compounds investigated the effect on the pseudocholinesterase activity and the seminal vesicle became smaller when the interval was increased from one to two days and so on.

2. The effect on the levator ani showed very little change at intervals of up to four days.

3. With an injection interval of four days when TPP still has a relatively potent effect on the enzyme activity and on the seminal vesicle, T is almost completely inactive (N has no noteworthy effect at any dose level).

These observations suggest that a »prolonged action« may indeed be the cause of the more potent effect of NPP. As to the question whether this is due to differences in absorption rate, differences in distribution or differences in elimination rate, it is of interest to refer to the paper of van der Vies (1965), who investigated the mechanism of action of NPP. From his investigations he concluded that both the duration of action and the relative effects on levator ani muscle and seminal vesicle depend on the rate at which they are absorbed from their depot. The fact that the effects on the levator ani are less sensitive to changes in the injections, may be explained either by a stronger binding of the hormones to the muscle cells, or a more prolonged reaction to the hormone stimulus.

REFERENCES


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