SEXUAL DIFFERENCE IN SUSCEPTIBILITY TO CARDIAC ARRYTHMIAS INDUCED BY ADRENALINE

By

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In 1952 Astarabadi et al. reported a difference in susceptibility to toxic doses of adrenaline between male and female rats, the resistance of the females being higher than that of the male rats. One month after bilateral oophorectomy, however, the females became as sensitive as the males. Contrary to these findings Johnstone (1953) reported, that cardiac arrhythmias, induced by adrenaline in patients under cyclopropane anaesthesia and who had been premedicated with atropine sulphate, occurred more readily in females than in males. We thought it of interest to study this striking difference in sex-linked susceptibility to toxic effects of adrenaline more closely.

MATERIAL AND METHODS

The experiments were performed with normal adult male and female guinea pigs, weighing 500–700 gm. and with normal adult rats of an inbred strain of the Pharmaco-Therapeutic Laboratory of the University of Amsterdam, weighing from 275 to 325 gm. The guinea pigs as well as the rats were divided into three groups: normal males, normal females and male animals implanted subcutaneously with pellets of 25 mg. oestradiol benzoate. The guinea pigs received two and the rats one pellet seven days before the experiment.

The guinea pigs were anaesthetized by subcutaneous injection of urethane (1 gm./kg. body weight), dissolved in saline, followed after one hour by 15–20 mg. of pentobarbitone/kg. body weight given intraperitoneally. The rats were anaesthetized in the same way, except that the pentobarbitone was administered 30 minutes after the injection of urethane. Artificial respiration was given to the guinea pigs in most of the

Blood pressure recordings of a \( \varphi \) rat.

A. Irregular respiration: the separate heart beats are clearly visible during sharp falls in blood pressure.

B. Arrhythmias of the heart: no separate heart beats visible during sharp falls in blood pressure.

Experiments. All the rats were allowed to breathe spontaneous throughout the experiment.

To avoid blood clotting, all guinea pigs and rats were injected with 1 mg. of heparin (Organon N. V.) in 1 ml. saline intravenously. Blood pressure was recorded by means of a double membrane tambour (cf. d'Amour et al., 1948). This method of recording, although less convenient for direct blood pressure registration than a mercury manometer, allows of the registration of the separate cardiac pulsations. Due to the high sensitivity of the apparatus, acute changes in blood pressure caused by extra systoles are easily distinguished from changes following irregularities in the respiration (see Fig. 1). As arrhythmias of the heart, recorded by electrocardiographic registration closely correspond to the irregularities seen in the blood pressure recordings, we decided to use the latter recordings only. Adrenaline (Brocappharm) was administered through a canula inserted into the external jugular vein. In the experiments with guinea pigs, treatment with adrenaline was started with a dose of 2 \( \mu g \). This dose was increased by 2 \( \mu g \) at a time until a dose was reached which caused arrhythmias. In the experiments with rats, we started with 0.2 \( \mu g \) of adrenaline followed by 0.4 \( \mu g \), 0.6 \( \mu g \) until a dose of 1.2 \( \mu g \) was reached. In the latter experiments each dose was administered three times in succession. No further injection was given until the blood pressure had been normal again for a few seconds. The adrenaline, used in the experiments both in the guinea pigs and rats was dissolved in saline. All doses were injected in a standard volume of 0.5 ml.

**RESULTS**

A. Guinea pigs. In these experiments the threshold dose of adrenaline which caused irregularities of the heart was determined. The results are summarized in Table 1.
Table 1.
The threshold intravenous dose of adrenaline causing cardiac arrhythmias in normal and oestrogen implanted guinea pigs.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Treatment</th>
<th>Number of animals</th>
<th>Threshold dose of adrenaline in μg. mean ± s. e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂</td>
<td>—</td>
<td>14</td>
<td>11.6 ± 2.65</td>
</tr>
<tr>
<td>♀</td>
<td>—</td>
<td>6</td>
<td>39.7 ± 14.2</td>
</tr>
<tr>
<td>♂</td>
<td>2 × 25 mg. oestradiol benzoate pellets</td>
<td>9</td>
<td>18.1 ± 5.58</td>
</tr>
</tbody>
</table>

From these results it is obvious that the dose causing irregularities in males is considerably smaller than in females, this difference being statistically significant according to Wilcoxon's test (P₂ < 0.01). Due to considerable variations of the data, the difference between oestradiol-treated and untreated male animals is not significant (P₂ = 0.6). A group of eight male guinea pigs used in our next experiments were in a rather poor condition. As these animals proved to be very sensitive to adrenaline – all showing severe arrhythmias to doses of adrenaline of ≤ 2 μg. – it was suspected, that seasonal differences in body condition might interfere too much with the results of our experiments. We therefore decided to continue the experiments with rats, these animals being in good condition throughout the year.

B. Rats. ² For these experiments we used three groups of rats, viz. 23 untreated males, 23 untreated females and 15 male rats, implanted subcutaneously with one pellet of 25 mg. of oestradiol benzoate. The threshold doses of adrenaline, causing cardiac arrhythmias are summarized in Table 2.

Table 2.
The threshold intravenous dose of adrenaline causing cardiac arrhythmias in normal and oestrogen treated rats.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Treatment</th>
<th>Number of animals</th>
<th>Threshold dose of adrenaline in μg. mean ± s. e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>♂</td>
<td>—</td>
<td>23</td>
<td>0.68 ± 0.08</td>
</tr>
<tr>
<td>♀</td>
<td>—</td>
<td>23</td>
<td>0.91 ± 0.06</td>
</tr>
<tr>
<td>♂</td>
<td>1 pellet of 25 mg. of oestradiol benzoate</td>
<td>15</td>
<td>0.92 ± 0.09</td>
</tr>
</tbody>
</table>

² Most of the experiments with rats were performed by Mr. W. S. H. van Drooge with the technical assistance of Mr. J. C. van Houten (Pharmaco-Therapeutic Laboratory of the University of Amsterdam). We should like to express our thanks for their valuable contribution to this investigation.
Application of the test of Wilcoxon revealed that the differences both between normal male and female rats and between oestrogen treated and untreated male rats were significant, \( P < 0.02 \) and \( < 0.01 \) respectively.

In this series of experiments every dose of adrenaline was given three times in succession and all animals received from 0.2 \( \mu \text{g.} \) up to 1.2 \( \mu \text{g.} \) of adrenaline. This method allowed us to calculate in the three groups of animals, for each dose of adrenaline, the percentage of the total number of injections which gave rise to cardiac arrhythmias. Fig. 2 shows the effect of the doses of adrenaline used in the three groups of animals.

Since we used one male and one female rat on each experimental day and in alternate order we were able to apply the »sign-test« to our results. The
difference found between males and females at a dose level of 0.8 µg. adrenaline proved to be significant \( P_2 = 0.04 \).

**DISCUSSION**

Our experiments both with guinea pigs and rats show a distinct difference in sensitivity to cardiac arrhythmias induced by adrenaline between male and female animals. Although the experiments with male guinea pigs implanted with oestradiol pellets were not convincing, the experiments with oestradiol implanted into male rats indicate that the presence of oestrogens is most probably responsible for this higher resistance in female animals. Our results are in agreement with the work of Astarabadi et al. (1952) who showed that the lethal dose of adrenaline for female rats was higher than that for male rats. Johnstone (1953), however, working with patients under cyclopropane anaesthesia, found that after pretreatment with atropine sulphate, female subjects were more susceptible to cardiac arrhythmias induced by adrenaline than male patients. To understand this divergence in results it will be necessary to analyse the mechanism by which oestrogens influence the development of cardiac arrhythmias by adrenaline. As early as 1914 Nobel et al. stressed the importance of the vagal reflex in the induction of cardiac arrhythmias by adrenaline. Later Petzetakis et al. (1930), Wilburne et al. (1947) and others (cf. Riker et al., 1955) held the same opinion.

Other investigators, however, using atropine or bilateral vagotomy to abolish vagal depressor activity during adrenaline reactions, observed little or no protection from these procedures (cf. Nickerson, 1947, Nickerson et al., 1947). Nickerson et al. (1949) investigating the mechanism of protection by dibenamine against cardiac arrhythmias induced by adrenaline in animals under cyclopropane anaesthesia, stated that the absolute rise in blood pressure after adrenaline is of great importance in the production of the irregularities.

Recent work by Riker et al. (1955) seems to provide an explanation for this discrepancy. In their experiments with cats they demonstrated a difference between arrhythmias induced in animals under allobarbitone anaesthesia by adrenaline alone, and arrhythmias induced by a combined treatment of adrenaline together with inhalation of hydrocarbons. While pretreatment with atropine or bilateral vagotomy results in a protection in the former type of cardiac irregularities, these treatments have no significant effect when hydrocarbons are used in addition to adrenaline. This is in agreement with the results of other workers. Wilburne et al. (1947) also observed the protective effects of atropine treatment, when working with unanaesthetized dogs, though other investigators observed no effect from vagotomy or atropine treatment in animals under cyclopropane anaesthesia (Nickerson, 1947, Nickerson et al., 1947).

Since hydrocarbons exert an unspecific depressor action on the heart muscle
(Riker et al., 1955) which is neither influenced by atropine or by bilateral vagotomy, the difference in the protective effect of these treatments on the arrhythmias produced by either adrenaline or the combination of hydrocarbons and adrenaline is readily understood.

If Nickerson's hypothesis on the significance of the absolute blood pressure rise for the occurrence of arrhythmias (Nickerson et al., 1947) were true, oestrogens should diminish the absolute blood pressure rise after the injection of adrenaline. However, Boxill et al. (1955) found that intact female dogs show a higher blood pressure response to the injection of adrenaline than male dogs. Their report is in agreement with the previous work of Woodbury et al. (1947) and Ahlquist et al. (1954).

We conclude therefore, that oestrogens can exert their protective effect against the induction of cardiac arrhythmias by adrenaline in one of the following ways:

a) A direct protective influence on the myocardium.

b) Depression of vagal activity.

c) Influence of oestrogens on pentobarbitone anaesthesia.

This latter point is of interest, as some barbiturates are known to depress vagal activity (Goodman et al., 1955).

Further experiments on these lines are in progress.

SUMMARY

1. Female rats and guinea pigs under a combined urethane-pentobarbitone anaesthesia are more resistant against the induction of cardiac arrhythmias by adrenaline, than male animals of the same species.

2. Implantation of one pellet of 25 mg. of oestradiol benzoate subcutaneously into normal adult male rats increases their resistance to about the level of the female rats.

3. The mechanism by which oestrogens may influence the development of cardiac arrhythmias induced by adrenaline is discussed.

ACKNOWLEDGMENT

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REFERENCES
