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Hormonal regulation of rat seminal vesicle sensitivity to adrenaline, noradrenaline, acetylcholine and acetyl-β-methylcholine

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1. The influence of the hormonal condition of rats on the responsiveness of their seminal vesicles to drugs was investigated by determining pD_2 and α values for adrenaline, for noradrenaline, for acetylcholine and for acetyl-β-methylcholine on isolated vesicles of normal, of castrated, and of oestradiol-treated castrated rats.

2. pD_2 values of adrenaline, of noradrenaline, of acetylcholine and of acetyl-β-methylcholine acting on seminal vesicles of castrated or of oestradiol-treated castrated rats were greater than the corresponding values obtained on the organs of normal rats. The differences were significant at the level $P<0.001$.

3. Similar results were obtained for the relative intrinsic activity of noradrenaline and acetyl-β-methylcholine.

4. Consequently affinity of adrenaline, noradrenaline, acetylcholine and of acetyl-β-methylcholine for their corresponding receptors and the stimulus produced by each unit of the noradrenaline-receptor or acetyl-β-methylcholine-receptor complex were enhanced.

5. The data indicate that an increase in the sensitivity of the smooth musculature of seminal vesicles to adrenaline and noradrenaline as well as to acetylcholine and acetyl-β-methylcholine is obtained by castration, or by treatment with oestradiol following castration.

Waddell (1917) seems to have been the first author to study the pharmacology of seminal vesicles of rats and guinea-pigs. He concluded that the seminal vesicles have both sympathetic and parasympathetic innervation. Later, the behaviour of genitals of male rats (Wilcke, 1937 ; Martins & Valle, 1939 ; Martins, Valle & Porto, 1939a ; Valle & Picarelli, 1954), cats (Martins & Valle, 1940), rhesus monkeys (Martins, Valle & Porto, 1939b), rabbits (Porto, 1943), guinea-pigs (Valle & Porto, 1939), and dogs (Valle & Porto, 1947), and the responses of these organs to various drugs, were shown to be dependent on the hormonal state of the donor animals. In general, the seminal vesicle, the vas deferens, and the prostate of the castrated rat contracted spontaneously and rhythmically ; organs from normal or testosterone-treated castrates were quiescent. Most of the drugs used, when acting

on organs taken from castrates or from castrates treated wih oestrogen, produced an increase in tonus followed by the appearance of rhythmic contractions. When these same drugs acted on organs of normal or of castrated animals treated with testosterone, the only response was an increase in tonus. Furthermore, organs from castrates and from oestrogen-treated castrates seemed to present a greater sensitivity to the drugs.

This last aspect of the problem was dealt with by Grunt & Higgins (1960) in their determinations of the threshold doses of adrenaline, noradrenaline, and acetylcholine on the isolated seminal vesicle of the rat. These investigators concluded that vesicles of castrates had lower thresholds to adrenaline, and possibly to acetylcholine, but not to noradrenaline. The thresholds to acetylcholine, but not to noradrenaline, were higher when water-soluble androgen was added to the isolated preparation.

Because determination of threshold doses is not the most suitable method for evaluating changes in sensitivity of pharmacological preparations (Trendelenburg, 1963), the work described below was undertaken in an effort to approach the problem more quantitatively.

Methods

Seminal vesicles were excised from normal (275–310 g), castrated (275–340 g), and oestradiol-treated castrated (250–310 g) adult albino rats. Castration was performed under ethyl ether anaesthesia 30 to 50 days before using the animals; hormonal treatment was given by subcutaneous injection of 0.5 mg of oestradiol benzoate to each rat every 48 hr beginning with the day of castration.

After animals were killed with ethyl ether, one seminal vesicle was removed, separated from the coagulating gland, and freed of its secretion by several washings with physiological solution of the following composition (mM): NaCl 136, KCl 5.6, $CaCl_2$ 1.8, NaH_2PO_4 0.36, $NaHCO_3$ 15.0 and dextrose 5.5; pH=7.6. The organ was then suspended in a 10 ml. tissue chamber containing the nutritive solution, aerated, and maintained at 30° C. Longitudinal contractions were recorded with the aid of a frontal isotonic lever giving a sixfold magnification with a load of 0.5–1.0 g.

Dose-effect curves were obtained by the cumulative technique (van Rossum, 1963). Molar concentration of the drugs was increased geometrically with a progression ratio of 3.2. In general, three dose-response curves for adrenaline and three for noradrenaline were obtained alternately on each preparation. A resting period of 30 min was allowed between two consecutive curves. This same procedure was followed for acetylcholine and acetyl-β-methylcholine.

Height of contraction produced by each molar concentration, calculated as a percentage of the maximum effect of adrenaline (for the sympathomimetics) or of acetylcholine (for the parasympathomimetics) was plotted against the logarithm of the corresponding concentration. Values for pD_2, the negative logarithm of the drug concentration producing 50% of its maximum effect (Miller, Becker & Tainter, 1948), were obtained by interpolation. Relative intrinsic activity α (Ariens, 1954) was calculated as the ratio between the maximum effects of the drugs. The mean values of pD_2 and α obtained in each experiment were calculated, and the data obtained with the three groups of animals were submitted to analysis of variance (Emmens, 1948).

Drugs used in these experiments were: (−)-adrenaline and (−)-noradrenaline bitartrate (Winthrop-Stearns, Inc.), acetylcholine and acetyl-β-methylcholine chloride (Sigma Chem. Co.).

Results

Examples of dose-response curves obtained with adrenaline and noradrenaline on seminal vesicles excised from normal, from castrated, and from oestradiol-treated castrated rats are shown in Fig. 1. The two latter types of vesicles contracted rhythmically, but the maximum heights reached by them in response to both drugs were smaller than the maximum effects elicited from the vesicles of normal rats. A similar phenomenon was observed with acetylcholine and acetyl-β-methylcholine.

When the logarithms of the molar concentrations of adrenaline or noradrenaline are plotted against the corresponding mean effects of the drugs, calculated as percentage of the maximum effect produced by adrenaline on each type of preparation, the curves differ (Fig. 2). Taken as the standard drug, adrenaline gave a uniform maximal effect; noradrenaline gave different maxima. Although on vesicles of normal rats the noradrenaline maximum was lower than that of adrenaline, on the two other vesicle preparations, the opposite result was obtained.

Figure 3 shows the results obtained for acetylcholine and acetyl-β-methylcholine. On vesicles of normal rats, the maximal effect of acetyl-β-methylcholine was lower than that of acetylcholine but on vesicles of castrated or oestradiol-treated castrated rats it was greater.

These differences in maximal effects will be responsible for different values of the relative intrinsic activities α of noradrenaline or of acetyl-β-methylcholine, in the three types of preparation.

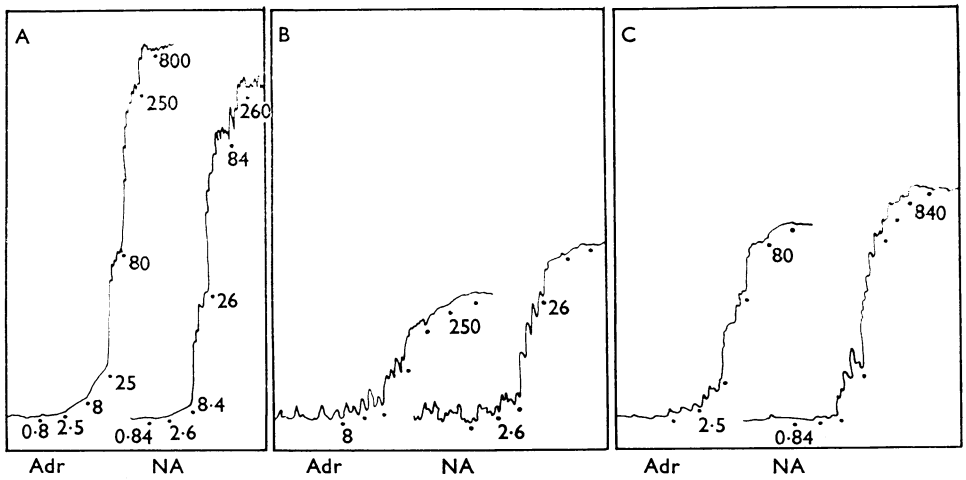

FIG. 1. Cumulative dose-response curves for adrenaline (Adr) and noradrenaline (NA) obtained on the isolated seminal vesicles of normal (A), of castrated (B), and of oestradiol-treated castrated (C) rats. Figures indicate final concentration of the drugs ($\times 10^{-4}$ mM) in the organ bath; it increased geometrically with a progression ratio of 3.2. Rhythmic contractions appeared on the vesicles of castrated rats. Maximal effects produced by both drugs on vesicles excised from castrated or from oestradiol-treated castrated rats were smaller than those obtained on organs taken from normal rats.

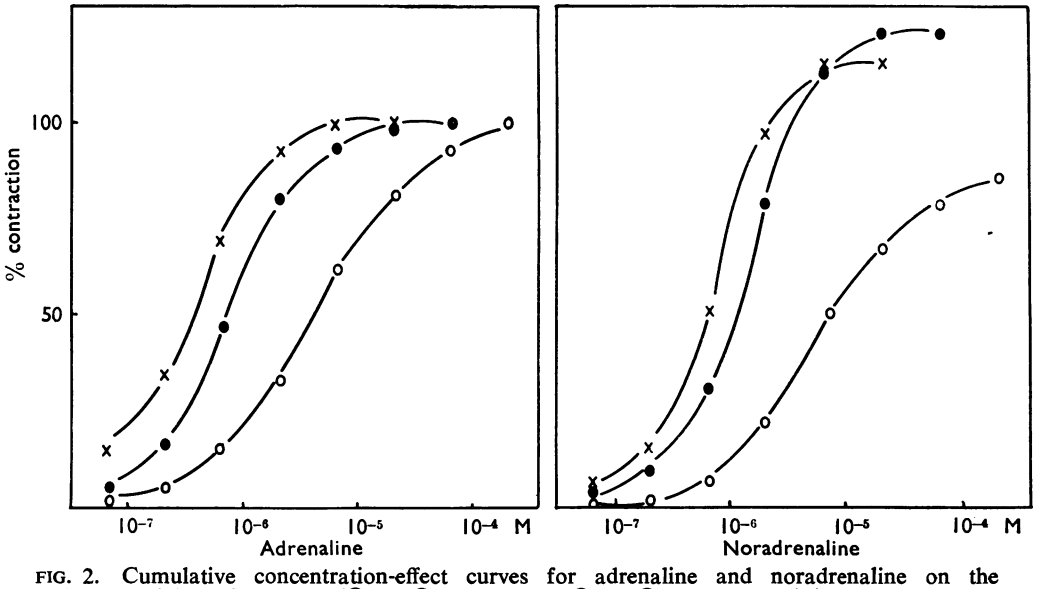

FIG. 2. Cumulative concentration-effect curves for adrenaline and noradrenaline on the isolated vesicles of normal (○——○), castrated (●——●), or oestradiol-treated castrated (×——×) rats. Abscissa: molar concentration of the drugs, on a logarithmic scale. Ordinate: effects as percentage of the maximal contraction of adrenaline. There is a shift to the left of two of the curves obtained for adrenaline and for noradrenaline, leading to changes in pD_2 values of both drugs. The differences in the maximal effects of noradrenaline will be responsible for different relative intrinsic activity values.

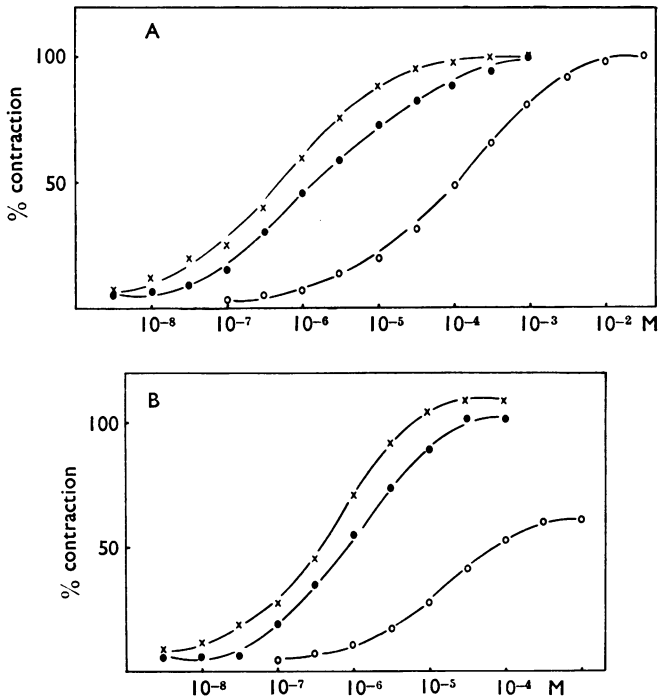

FIG. 3. Cumulative concentration-effect curves for acetylcholine (A) and acetyl-β-methylcholine (B). Same symbols as in Fig. 2. Abscissa: molar concentration of the drugs, on a logarithmic scale. Ordinate: effects as percentage of the maximal contraction of acetylcholine. The shift to the left is more pronounced than that observed in Fig. 2. Different pD_2 values for both drugs and different α values for acetyl-β-methylcholine will result.

TABLE 1. *Affinity and intrinsic activity of adrenaline, noradrenaline, acetylcholine and acetyl-β-methylcholine (mean and corresponding standard error) on isolated seminal vesicles of rats in different hormonal conditions*

Drugs / group of rats	Affinity pD_2			Relative intrinsic activity α		
	Normal	Castrated	Oestradiol-treated castrated	Normal	Castrated	Oestradiol-treated castrated
Adrenaline	5·33±0·06 (12)	6·08±0·04 (14)	6·38±0·05 (10)	1·00	1·00	1·00
Noradrenaline	5·18±0·06 (12)	5·73±0·04 (14)	6·03±0·04 (10)	0·87±0·02	1·25±0·04	1·17±0·03
Acetylcholine	3·96±0·15 (11)	5·86±0·20 (10)	6·30±0·14 (12)	1·00	1·00	1·00
Acetyl-β-methylcholine	4·89±0·17 (11)	6·06±0·19 (10)	6·35±0·13 (12)	0·61±0·04	1·08±0·07	1·09±0·02

Differences in the mean values of pD_2 or α for the three groups of rats were shown by variance analysis to be significant at the level $P<0·001$. Number of experiments in brackets.

Figures 2 and 3 also show that there is a shift to the left in the dose-effect curves depending on the hormonal state of the rat from which the vesicles were excised. This phenomenon is more pronounced for acetylcholine and acetyl-β-methylcholine. As a consequence of this shift, the concentrations of adrenaline, noradrenaline, acetylcholine or acetyl-β-methylcholine producing 50% of their maximal effects on vesicles from both castrated and oestrogen-treated castrated animals will be smaller. This will cause an increase in the values for pD_2 of the four drugs.

The means and corresponding standard errors, calculated from the values of pD_2 and α determined for each experiment with the three different types of vesicles, are presented in Table 1. Besides the increase in the values of pD_2 of the four drugs it may be noticed that the relative intrinsic activities α of noradrenaline or of acetyl-β-methylcholine were greater when determined on vesicles of castrated or oestradiol-treated castrated rats.

By analysis of variance, the observed differences in pD_2 as well as in α values were significant at a level of $P<0.001$.

Discussion

In their attempt to study quantitatively the interrelations between rat hormonal state and responsiveness of the seminal vesicles to drugs, Grunt & Higgins (1960) confirmed that vesicles of castrated rats contracted spontaneously and rhythmically and that vesicles from intact animals never showed such behaviour. Although small changes in the resting conditions of the pharmacological preparations could lead to gross errors in the determination of the threshold dose, they stated that the sensitivity of seminal vesicles to acetylcholine, adrenaline and noradrenaline was affected differently by castration or androgen-treatment of the rats from which the vesicles were excised. This statement was not in accordance with the experiments reported by Martins and his group showing that castration or oestrogen-treatment always raised, and androgen-treatment always lowered the sensitivity of the male genital organs to drugs, mainly to parasympathomimetics.

It was therefore thought worth while to determine dose-response curves of some drugs on vesicles of rats in various hormonal states and to analyse variations in sensitivity by the horizontal shifts in such curves.

The results demonstrate that two constants, pD_2 and α, characteristic of the drugs, vary according to the type of seminal vesicle from which they are derived. Castration and oestradiol-treatment following castration induce an increase in pD_2 values of adrenaline, noradrenaline, acetylcholine and acetyl-β-methylcholine as well as in α values of noradrenaline and acetyl-β-methylcholine, when compared with the corresponding values determined on the same organs from normal donors.

Because pD_2 values are correlated with affinity of drugs for their receptors, it may be inferred that affinity of the four drugs for their respective receptors in seminal vesicles, varies according to the hormonal state of the donor rat. There is also an increase in the relative intrinsic activities α of noradrenaline and acetyl-β-methylcholine, so it can be assumed that, at least for these two drugs, the stimulus produced by each unit of the drug-receptor complex, which is measured through α, is enhanced.

Both increases in affinity and in relative intrinsic activity will contribute to a greater sensitivity to the drugs tested of vesicles taken from rats submitted to

castration alone or to castration followed by treatment with oestradiol. These results point to a difference in the localization or in the relative concentrations of adrenoceptive receptors in the rat seminal vesicles under the influence of oestradiol when compared with cat vas deferens (Martins & Valle, 1940) or guinea-pig seminal vesicles (Valle & Porto, 1939), in which adrenaline may exert an inhibitory action when the animal has been treated with oestrogens. Gonadal status of the rats influenced not only the responses to adrenaline and acetylcholine but also to noradrenaline, so this implies a probable interaction between peripheral autonomic nervous system and hormones. In which way and to what extent either absence of testosterone or presence of oestradiol can facilitate the interaction between sympathomimetic and parasympathomimetic drugs and their receptors at the level of the smooth muscle of male genitals is, up to now, a matter for speculation.

Though maximal effects of the drugs on the vesicles of castrated and of oestradiol-treated castrated rats were smaller than those obtained on organs of control rats (Fig. 1), the range of doses covering the dose-response curves on those organs was lower. For acetylcholine, this range of doses varied from 10^{-7} to 10^{-2}M on organs taken from normal rats and from 3×10^{-9} to 10^{-4}M on organs taken from castrated or oestradiol-treated castrated rats. Though isotonic records of the type used in this study are not the most appropriate to measure strength of contraction, a possible explanation for the smaller maximal effects observed may be that after castration the seminal vesicles possess a low contractile power. Such an assumption is reasonable in view of the atrophy of the organs observed after castration. Csapo & Corner (1953) have already shown that strength of contraction is dependent on muscular actomyosin concentrations and that there is a direct relation between this level and oestrogen concentration in rabbit uterine muscle. This correlation could be the reason for the slight increase in maximal effects of adrenaline, noradrenaline, acetylcholine and acetyl-β-methylcholine observed on the vesicles of oestradiol-treated castrates, when compared with those produced on organs excised from castrated rats.

The present observations need to be extended by recording isometric contractions of the three groups of seminal vesicles exposed to the action of the same drugs. Data regarding the effects of specific antagonists of the drugs and analysis of the *in vitro* influence of water soluble oestrogens, androgens or progesterone on the behaviour of isolated vesicles of normal or castrated rats will shed more light on the mechanism of action of these hormones at the end organ level.

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