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# THE USE OF THE RAT'S ISOLATED SEMINAL VESICLE FOR THE ASSAY OF SYMPATHOLYTIC DRUGS*

BY

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The isolated seminal vesicle of the guinea-pig has been used by Brugger (1945), Rothlin and Brugger (1945), and Rothlin (1947) for the evaluation of the sympatholytic effect of the natural and hydrogenated ergot alkaloids; by Stone and Loew (1952) for the assay of sympatholytic (anti-adrenaline) drugs; and by Meier (1950) for the study of antihistaminic drugs. To the best of our knowledge, no information is available on the comparable use of the isolated rat seminal vesicles, although they have been used recently by Haley and Leitch (1954) in an attempt to detect ferritin inhibition of adrenaline responses. The present paper deals with the reaction of the rat's isolated seminal vesicles to adrenaline, acetylcholine, histamine, and a series of anti-adrenaline drugs.

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## METHODS

The seminal vesicles and associated structures were removed from Wistar strain rats of 250 to 500 g. and placed in Ringer-Locke solution of the following composition: NaCl, 9.0 g.; KCl, 0.42 g.; $CaCl_2,2H_2O$, 0.24 g.; $MgCl_2,6H_2O$, 0.005 g.; $NaHCO_3$, 0.5 g.; anhydrous glucose, 0.5 g.; and distilled water to 1 l.

Greene (1935) reported that the rat's seminal vesicles are contained within the same connective tissue sheath as the coagulation glands. It was found that the latter glands must be carefully removed, without injury to the vesicles, otherwise abnormal responses and reduced sensitivity result. After removal of all extraneous tissue, both vesicles were transected distally to their point of entrance into the ductus deferens.

Both preparations were transferred to the same tissue chamber and attached for dual recording. A 15 ml. tissue chamber, maintained in a water bath at 37.5± 0.2° C., and having a continuous flow of 100 ml. of Ringer-Locke solution per minute, was used in all experiments. Flow was interrupted when drugs were added to the chamber. The bathing solution was continuously oxygenated with a mixture of 95% $O_2$ and 5% $CO_2$. All drug dilutions were prepared in 0.9% NaCl and given in a standard volume of 0.5 ml. Preliminary tests showed that ten minutes after the seminal vesicles were placed in the tissue chamber, 30 μg. doses of adrenaline, given at six-minute intervals, produced responses which varied only slightly during the next two hours.

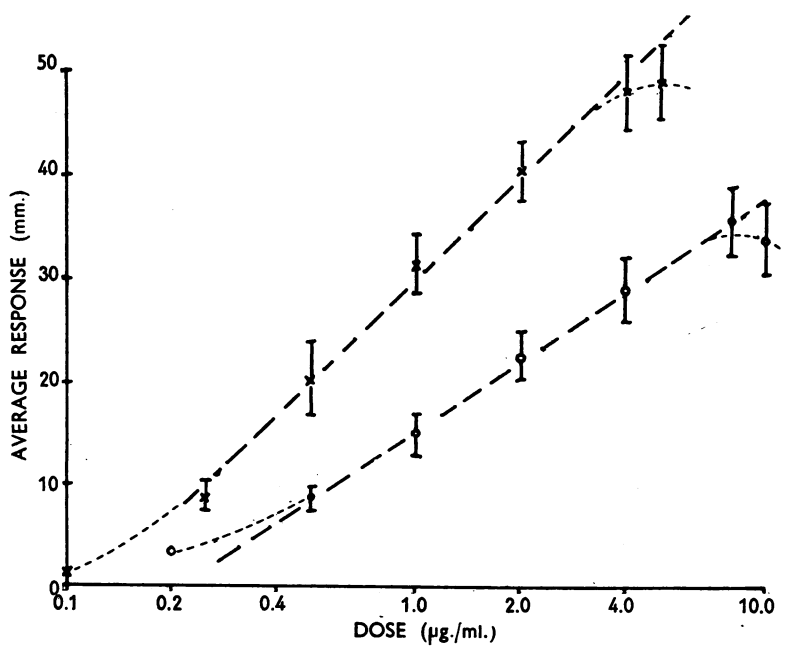


FIG. 1.—Response of rats' seminal vesicles to adrenaline (×) and acetylcholine (O).

In testing sympatholytic drugs, 30 μg. doses of adrenaline were given at six-minute intervals until the last two responses did not vary by more than ±5% from their average. Six minutes later the sympatholytic drug was given and allowed to act for one minute before the standard dose of adrenaline was repeated. Only one dose of blocking drug was given per tissue preparation because of the varying degrees of tissue fixation. However, piperoxane, which can be removed by washing, was alsot ested by giving multiple doses to the same preparation. Three additional doses of adrenaline were given after the blocking drug in order to obtain information on the degree of tissue fixation and the rate of elution of the latter. The results obtained in these experiments were statistically analysed by the Litchfield-Wilcoxon method (1949).

## Results

*Response to Adrenaline, Acetylcholine, and Histamine.*—The response to both adrenaline and acetylcholine was linear within the dosage range studied (Fig. 1). It appears, however, that there is a maximum for each drug, and that this tissue is more sensitive to adrenaline than to acetylcholine. No tissue fixation occurred with either of these drugs, and recovery of sensitivity was complete after washing for one minute. The rat seminal vesicle shows the same insensitivity to histamine as do other tissues of this species; doses of histamine as high as 2.2 mg. induced no contractions.

*Evaluation of Sympatholytic Activity.*—A comparison of the potencies of various sympatholytic drugs is given in Table I. There is no significant difference in the potency of piperoxane when evaluated by the single dose and by the multiple dose methods. Furthermore, ergotamine does not differ significantly in potency from piperoxane. Potency ratio comparisons, based on piperoxane as standard, show the following order of decreasing potency: "Dibenzyline," "Dibozane," "Sy-28," "Hydergine," and ergotamine.

*Tissue Fixation of Sympatholytic Drugs.*—There is variability in the degree of tissue fixation of the various sympatholytic drugs when they are given at their approximate ED50's and then tested every six minutes (Fig. 2). Drugs from the same chemical series show a similar degree of tissue fixation; the 2-haloalkylamines appear to attach themselves strongly to tissue receptors, whereas the benzo-

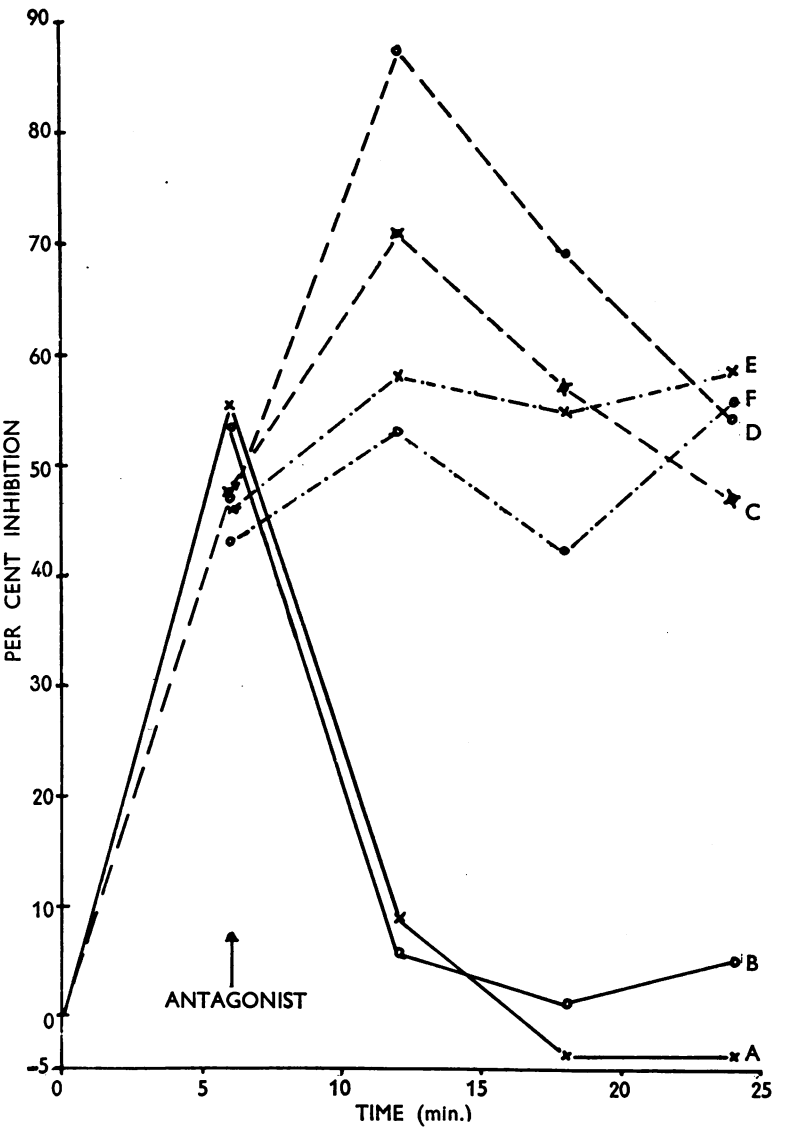


FIG. 2.—Tissue fixation of sympatholytic agents. A, Dibozane 0.5 μg. B, Piperoxane 6.0 μg. C, Hydergine 2.0 μg. D, Ergotamine 3.0 μg. E, Dibenzyline 0.1 μg. F, Sy-28 1.0 μg.

TABLE I
COMPARISON OF SYMPATHOLYTIC ACTIVITY ON RAT'S ISOLATED SEMINAL VESICLE

| Drug | Dosage Range μg./15 ml. | ED50 and Range* μg./15 ml. | Slope and Range* | Potency Ratio and Range* μg./15 ml. |
|---|---|---|---|---|
| Piperoxane hydrochloride 2-(1-piperidylmethyl)-1, 4-benzodioxane HCl | 2–10 | 4·85 (2·98–7·91) | 2·43 (1·07–5·84) | Standard |
| Piperoxane hydrochloride† | 1–75 | 5·15 (2·59–10·3) | 3·05 (1·65–5·64) | Equivalent to standard |
| Ergotamine methanesulphonate | 2–5 | 3·00 (2·42–3·72) | 1·74 (1·15–2·62) | ,, ,, |
| "Hydergine"‡ .. .. | 0·8–6·0 | 1·83 (1·15–2·92) | 3·28 (1·37–7·85) | >2·65 (1·35–5·2) |
| "Sy-28" *N*-(2-bromoethyl)-*N*-ethyl-1-naphthalenemethylamine HBr | 0·6–2·0 | 1·18 (0·8–1·74) | 2·5 (1·05–5·95) | >4·11 (2·2–7·65) |
| "Dibozane" 1, 4 bis(1, 4-benzodioxane-2-ylmethyl)-piperazine | 0·15–1·5 | 0·47 (0·29–0·75) | 3·38 (1·5–7·6) | >10·32 (5·2–20·5) |
| "Dibenzyline hydrochloride" *N*-(phenoxy*iso*propyl)-*N*-benzyl-2-chloroethylamine HCl | 0·05–0·2 | 0·098 (0·06–0·15) | 3·1 (0·97–9·9) | >49·5 (25·4–96·5) |

* All values at 95% confidence limits.
† Multiple dose per preparation experiment.
‡ An equi-mixture of dihydroergocornine, dihydroergocristine, and dihydroergokryptine.

dioxanes can be removed readily by washing. The ergot alkaloids occupy an intermediate position and they might have been removed from their tissue attachment by washing for a longer period.

## DISCUSSION

The relationship between log-concentration of acetylcholine and the response of the isolated rat seminal vesicle is similar to that obtained with the frog's isolated ventricle and rectus abdominis preparations (Clark, 1926, 1927). Such a relationship also exists for the response to adrenaline and is in accord with results obtained on the rabbit's isolated uterus (Gaddum, 1926) and the cat's nictitating membrane (Rosenblueth, 1932; and Bacq and Fredericq, 1935). A sigmoid curve always results although a good portion of this is essentially linear (Fig. 1). The type of curve obtained, and the rapid restoration of sensitivity to both acetylcholine and adrenaline on washing, suggests that a chemical reaction occurs between the drugs and surface receptors like that postulated for the frog's heart by Cook (1926), and for arterial strips by Wilkie (1928). The sensitivity of the rat's seminal vesicle preparation to both acetylcholine and adrenaline is within the same range as that of the guinea-pig's seminal vesicle (Brugger, 1945; Rothlin and Brugger, 1945; Meier, 1950; and Stone and Loew, 1952). The rat preparation has the advantage of being insensitive to histamine. This insensitivity could be exploited in the assay of mixtures containing all three drugs.

The potencies of the various sympatholytic agents as determined with the rat's seminal vesicle preparation agree favourably with those obtained by other procedures (Nickerson, 1949). However, the high potency of dibozane could not be predicted upon the basis of its chemical structure, because the related compound piperoxane had the lowest potency of all of the drugs evaluated. On the other hand, Marsh (1953) found dibozane to be one of the most potent sympatholytic drugs available.

In considering the degree of tissue fixation of sympatholytic agents (Fig. 2), it is necessary to consider tissue penetration, lipoid solubility, and direct chemical reactions with either tissue receptors or cell surfaces or both. The majority of cell membranes behave as though they are composed of two molecular layers of lipoid with a monomolecular layer of protein on either side (Davson and Danielli, 1943). The benzodioxanes have a greater water solubility than either the ergot alkaloids or the 2-haloalkylamines, and water solubility would be one of the factors determining the ease of removal from the cell surface in the absence of direct chemical combination with cell surface constituents. On the other hand, the lipophilic nature of the 2-haloalkylamines would assist their penetration of the cell membrane whereas their hydrophobic characteristics would delay their removal. However, the greatest factor determining tissue fixation is direct chemical reaction with tissue receptors and the 2-haloalkylamines have been shown to form immonium and vinyl intermediates which combine with cell constituents so as to give a prolonged blocking action (Nickerson and Nomaguchi, 1951). Such considerations would support the contention that the 2-haloalkylamines are firmly attached to the tissue receptors of the rat seminal vesicle, as illustrated in Fig. 2.

## SUMMARY

1. The rat's isolated seminal vesicle gives a linear log-dose response curve to both adrenaline and acetylcholine. It is more sensitive to adrenaline than to acetylcholine and is completely insensitive to large doses of histamine.

2. Sympatholytic potency can be estimated on the rat's seminal vesicle; the results agree with

those obtained by other methods. The following order of decreasing potency of the various drugs tested was obtained: " Dibenzyline," " Dibozane," " Sy-28," " Hydergine," ergotamine, and piperoxane.

3. It is possible to determine the degree of drug fixation on the tissue with the method. The 2-haloalkylamines have the greatest degree of tissue fixation and the benzodioxanes the least, with the ergot alkaloids occupying an intermediate position.

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