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The presence of β-adrenoceptors in the guinea-pig seminal vesicle

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Summary

1. The preparation of longitudinal smooth muscle strips from guinea-pig seminal vesicles is described.

2. Isoprenaline and salbutamol inhibited contractions produced by parasympathomimetic agents.

3. The inhibitory action of isoprenaline was blocked by low concentrations of propranolol and butoxamine. It was concluded that β_2-adrenoceptors were present in the tissue.

4. The inhibitory action of isoprenaline was not apparent when adrenaline, noradrenaline, hypogastric nerve or transmural stimulation were used to contract the tissue.

Introduction

The innervation and responsiveness to drugs of the guinea-pig seminal vesicles have been less extensively studied than is the case with the vas deferens. This is surprising because both tissues are supplied by the hypogastric nerve, and the vas deferens has proved to be a difficult tissue to use in some types of experiment. In particular, the vas deferens is relatively insensitive to drugs that cause a contraction of the smooth muscle unless certain procedures are carried out, such as stripping the outer coat from the organ (Bentley & Sabine, 1963) or opening the vas deferens along its length and scraping off the epithelium (Thoa & Maengwyn-Davies, 1968). Because of these inadequacies we have been studying the seminal vesicle preparation.

Guimarães (1969) has claimed that the seminal vesicle is devoid of β-adrenoceptors, and Davis (1971) used this preparation to analyse the interaction between different types of antagonists specifically because of the presumed absence of β-adrenoceptors. We have reinvestigated this question in guinea-pig seminal vesicles, and have found evidence for inhibitory β-adrenoceptors.

Methods

Male guinea-pigs weighing 350–1,050 g were killed and bled out. The seminal vesicles or vasa deferentia were rapidly removed and placed in McEwen's (1956) solution of the following composition (mM): NaCl 130, KCl 5·6, $CaCl_2$ 2·2, $NaHCO_3$ 25, NaH_2PO_4 1·2, glucose 11·1, and sucrose 13·2.

Preparation of longitudinal strips

The distal end of each vesicle was cut through and some of the seminal fluid gently expelled; then the mesentery, in which the hypogastric nerve runs, was removed. At this stage the two organs were separated and transferred to fresh McEwen's solution. Depending upon the size of the organs, either two or four strips were cut from the two structures. The first longitudinal cut was made along the line of attachment of the mesentery. In some cases the tissue was threaded over a short length of polythene tubing to facilitate the procedure. The sheet of smooth muscle was then transferred to fresh McEwen's solution, and was sometimes further divided. Strips were mounted in a 16 or 10 ml isolated organ bath.

Innervated seminal vesicle preparations

The whole seminal vesicle with attached hypogastric nerve was removed from guinea-pigs in a manner similar to that described by Naimzada (1966), and placed in a 100 ml organ bath. Care was taken not to cut or damage the mesentery through which the hypogastric nerve reaches the organ. The nerve was threaded through an electrode of the type described by Burn & Rand (1960) and stimulated from an electronic stimulator (Palmer, type H44). Trains of 200 pulses, each of 1 ms duration, were applied at 20 Hz every 2 min, the cycle being controlled by an electric motor. The output of the stimulator was set to give twice the voltage required to produce the maximal response of the tissue.

In experiments when the seminal vesicle was stimulated transmurally, an electrode similar to the one employed by Birmingham & Wilson (1963) was used. The stimulator was set to deliver pulses of 0·2 ms duration at 50 Hz for 4 s every 2 minutes. The maximum voltage output of the stimulator (100 V setting) produced responses that were consistent but could not be shown to be maximal.

The vas deferens–hypogastric nerve preparation

The dissection was similar to that described by Huković (1961). The conditions and stimulation characteristics of the tissue were identical with those described for the seminal vesicle–hypogastric nerve preparation.

Recording conditions

All preparations were placed in McEwen's solution at $32 \pm 0{\cdot}5°$ C, bubbled with 5% CO_2 in oxygen. Contractions were recorded on a kymograph by means of an isotonic frontal-writing lever, the magnification of which was eightfold. The load on the preparations was between 0·2 and 0·5 g; heavier loading gave unsatisfactory results. The preparations were not used within the first half-hour, but the bathing fluid was changed regularly.

Drugs used

The concentrations of drugs mentioned in the text refers to the forms listed below. The catecholamines were protected from oxidation by the inclusion of a small amount of ascorbic acid in each dilution.

(−)-Noradrenaline bitartrate (Koch-Light), (−)-isoprenaline sulphate (K & K

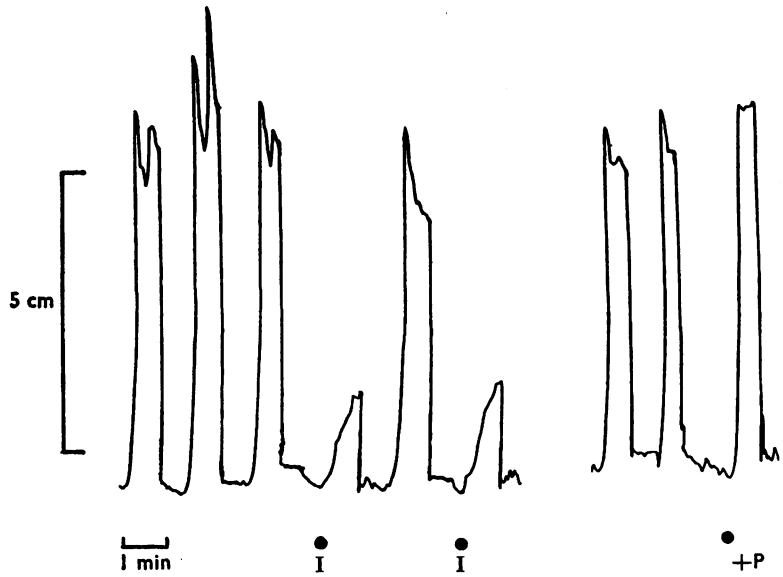

FIG. 1. The blockade of the inhibitory effect of isoprenaline by propranolol on the longitudinal strip of the guinea-pig seminal vesicle. All the contractions were produced by acetylcholine (11 μM). The panel on the left shows two control responses to isoprenaline (1·8 μM) plus acetylcholine at I, and the right panel depicts the blockade of the effect of isoprenaline by propranolol (1·7 μM at I+P) added 2 min before the catecholamine. The vertical calibration is 5 cm, and the contact time for acetylcholine was 1 minute. Note the exceptionally large effect of isoprenaline (see Fig. 2) and that propranolol completely blocked the inhibition.

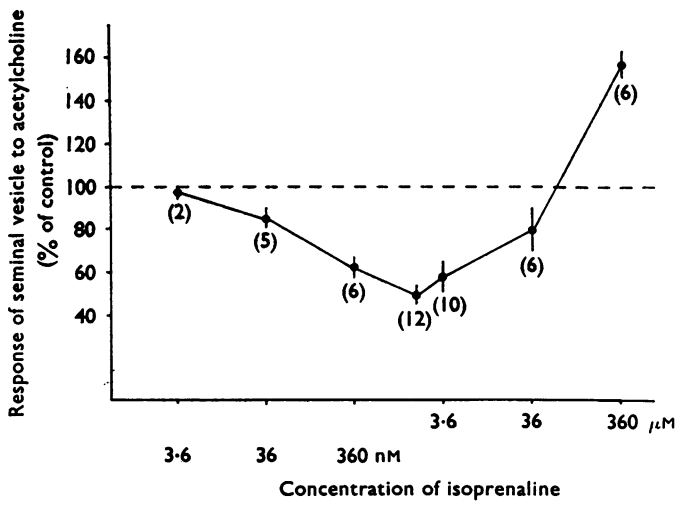

FIG. 2. The effects of isoprenaline on the contractions of the seminal vesicle longitudinal strip to acetylcholine (0·55–55·0 μM). The bars are the standard errors of the means, and the numbers in parentheses refer to the number of observations. Note that the inhibitory effect of isoprenaline reached a maximum at 1·8 μM, and that at 360 μM it increased the effects of acetylcholine.

Labs. Inc.), (−)-adrenaline base B.P. (Koch–Light: solution was effected with the minimum quantity of HCl), (±)-salbutamol micronized base (Allen & Hanburys), acetylcholine chloride (B.D.H.), methacholine chloride (Sigma), carbachol chloride (B.D.H.), (−)-propranolol hydrochloride (I.C.I. 47320), (±)-practolol base (I.C.I. 50172), (±)-butoxamine base (Burroughs Wellcome), phentolamine mesylate B.P. (Ciba), dihydroergotamine tartrate (Sigma), and (−)-ascorbic acid (B.D.H.).

Results

Effect of isoprenaline on responses to acetylcholine

Acetylcholine produced a rapid, vigorous contraction of the longitudinal strip of the seminal vesicle. In all experiments we selected a concentration of acetylcholine which caused consistent, submaximal contractions of the preparation. Low concentrations of isoprenaline introduced into the isolated organ bath 1 min before the next application of acetylcholine reduced the size of the contraction (Fig. 1). The effects of a range of concentrations of isoprenaline are depicted in Fig. 2. The maximum inhibition of 53 ± 3·6% (mean and standard error of the mean of 6 experiments) occurred at concentrations in the range 0·36–3·6 μM. Any further increase in the isoprenaline concentration reversed the trend, so that in the presence of 36–72 μM isoprenaline, the responses were as large as the controls. Even higher concentrations of isoprenaline augmented the acetylcholine-induced contractions. These curves were reminiscent of the ones obtained by Large (1965) on the guinea-pig vas deferens–hypogastric nerve preparation, which he described as ' inverted parabolic curves '.

It seemed likely that isoprenaline was acting on β-adrenoceptors to produce the inhibition of the contractions in response to acetylcholine, and that the augmentation could be due to the weak α-adrenoceptor stimulating property of isoprenaline (Bickerton, 1963 ; Large, 1965 ; Sutter, 1965 ; Gay, Rand & Wilson, 1967 ; Flacke, Osgood & Bendixen, 1967 ; Coupar, 1970 ; Day & Dixon, 1971 ; Eyre, 1971). In accordance with this, it was found that propranolol (1·7 μM) completely prevented the inhibitory action of isoprenaline (Fig. 1). Table 1 contains the results of a series of experiments in which the effects of the cardio-selective β-adrenoceptor blocking agent, practolol (Dunlop & Shanks, 1968), the extracardiac blocking agent, butoxamine (Levy, 1966) and propranolol have been compared. Butoxamine was more effective than practolol in blocking the inhibitory action of low concentrations of isoprenaline. This suggested that the effect of isoprenaline could be classified as an action on β_2-adrenoceptors (Lands & Brown, 1964 ; Lands, Arnold, McAuliff, Luduena & Brown, 1967 ; Lands, Luduena & Buzzo, 1967).

The augmentation of the acetylcholine responses with high concentrations of isoprenaline was difficult to study because the tissue did not readily relax after

TABLE 1. *Effect of β-adrenoceptor blocking agents on the inhibitory action of isoprenaline in the acetylcholine-stimulated longitudinal strip of the guinea-pig seminal vesicle. IC50 signifies the concentration of the antagonist which produced a 50% block of the inhibitory response to isoprenaline*

Antagonist	Individual IC50s μM	Mean IC50 μM
(−) Propranolol	0·068, 0·136, 0·034, 0·34, 0·34	0·17
(±) Practolol	>33·0 >66·0 >33·0	>33·0
(±) Butoxamine	3·7, 0·74, 1·48, 4·0, 2·2	2·5

Practolol exhibited some antagonism to the effects of isoprenaline in these experiments, but it did not reach 50% blockade.

such large contractions were produced. However, in a separate series of experiments, it was found that contractions of the strips occurred when an extremely high concentration (0·9 mM) of isoprenaline was applied on its own. In three experiments, maximal contractions, which represented about 60% of the maximum possible with noradrenaline, were obtained when 3·6 mM isoprenaline was added to the bath. In six further experiments, contractions produced by isoprenaline were reduced by dihydroergotamine (1·6 μM, two experiments) or phentolamine (5·3 μM, four experiments).

Effect of salbutamol

Salbutamol has been shown to have a relatively specific action on β_2-adrenoceptors (Cullum, Farmer, Jack & Levy, 1969), causing bronchodilatation with little cardiac action compared with isoprenaline (Palmer & Diament, 1969; Choo-Kang, Parker & Grant, 1970). Low concentrations (42 nM–4·2 μM) reduced acetylcholine-induced contractions of the seminal vesicle. The potency and maximal inhibition appeared to be similar to isoprenaline, though no direct comparisons were made. Salbutamol, however, did not augment the contractions when tested in high concentrations (0·42 mM), though the inhibitory effect was smaller than with lower concentrations.

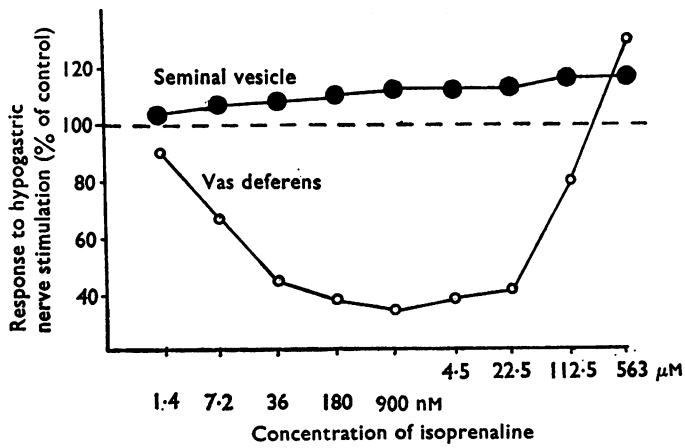

FIG. 3. A comparison of the effects of isoprenaline on the contractions of the seminal vesicle (●——●) and the contralateral vas deferens (○——○) to hypogastric nerve stimulation. Note that the inhibitory effect is clearly seen on the vas deferens but cannot be detected on the seminal vesicle.

TABLE 2. *The ability of various types of stimulation to reveal the inhibitory effects of isoprenaline on the longitudinal strip preparation of the guinea-pig seminal vesicles*

Agonist, or means of inducing contractions	Stimulation intensity, either concentration (μM) or rate (Hz)	Inhibitory effect of isoprenaline	No. of experiments
Acetylcholine	0·55–275·0	Present	37
Methacholine	0·51– 51·0	Present	8
Carbachol	0·55– 55·0	Present	8
Adrenaline	0·55–55·0	Absent	4
Noradrenaline	0·30–90·0	Absent	9
Hypogastric nerve stimulation	20	Absent	8
Transmural stimulation	50	Absent	5

Effect of isoprenaline on response to nerve stimulation and to noradrenaline

Low concentrations of adrenaline or isoprenaline inhibit the response of the vas deferens to hypogastric nerve stimulation (Ohlin & Strömblad, 1963; Holman & Jowett, 1964; Large, 1965; Ganguly & Bhattacharya, 1969). However, neither with nerve nor with transmural stimulation was it possible to demonstrate a reduction in the size of the contractions of the seminal vesicle when isoprenaline was added. Figure 3 shows an experiment in which the effects of isoprenaline on the contractions of the vas deferens and on the contralateral seminal vesicle were investigated. Both tissues were stimulated through their respective hypogastric nerves, but an inhibitory effect was detected only on the vas deferens.

From the evidence of Falck, Owman & Sjostrand (1965) and Wakade & Kirpekar (1971) it is known that the innervation of the guinea-pig seminal vesicle is noradrenergic. Thus, we thought it of interest to ascertain if isoprenaline produced an inhibitory effect in preparations which were stimulated by noradrenaline. No inhibition was found when either noradrenaline or adrenaline was used to contract the tissue. We have summarized in Table II the results obtained with isoprenaline when different methods were used to contract the seminal vesicles.

Discussion

Evidence is presented in this study which suggests that there are β-adrenoceptors in the seminal vesicle of the guinea-pig. Two types of experiment provided results that supported this conclusion. First, drugs noted for the selectivity of their action on β-adrenoceptors, isoprenaline and salbutamol, produced a diminution in the size of the contractions of the tissue to acetylcholine when they were used in low concentrations. Secondly, the inhibition of acetylcholine-induced contractions by isoprenaline was completely blocked by low concentrations of propranolol or butoxamine, and partially blocked by much higher concentrations of practolol. Dunlop & Shanks (1968) found that practolol had slight activity against the relaxation of isolated tracheal muscle by adrenaline, so it was not inconsistent that we have detected some antagonism with this drug in our experiments. The effectiveness of butoxamine suggests that β_2-adrenoceptors are present in the guinea-pig seminal vesicle.

At first it may appear surprising that Guimarães (1969) failed to detect the presence of β-adrenoceptors in this preparation. Guimarães used two criteria for their existence and neither was satisfied in his experiments. In the first place, he added isoprenaline when the contraction of the tissue to the stimulant drug was at its height, and failed to observe a relaxation of the smooth muscle. We have repeated this procedure and can confirm that isoprenaline does not unequivocally relax the seminal vesicle in this type of experiment. Guimarães (1969) also found that isoprenaline failed to inhibit contractions produced by subsequent addition of adrenaline, noradrenaline or phenylephrine. Our experiments with sympathomimetic agents have also failed to reveal an isoprenaline-induced inhibition. Thus, it appears necessary that tests for inhibitory β-adrenoceptors in isolated organs should allow for the addition of isoprenaline *before* the smooth muscle contracting drug, and that a range of spasmogenic agents should be used. It is of interest to note that Saxena (1970) did find evidence of β-adrenoceptors in the seminal vesicle using the anaesthetized guinea-pig. This author stimulated the hypogastric nerves

and measured the pressure in a balloon inserted inside the organ. In these experiments, it is possible that the β-adrenoceptors were easily detected because the circular muscle would contribute to the response. Alternatively, it could be another example of the contrasting pharmacological effects obtained in whole animal and isolated organ experiments.

The present results obtained with the isolated seminal vesicle contrast with those reported by Large (1965) for the associated organ, the vas deferens. With the vas deferens, isoprenaline inhibited contractions produced by nerve stimulation or noradrenaline, but not by adrenaline. Large (1965) suggested that adrenaline was probably combining with β-adrenoceptors as well as with the α-adrenoceptors which lead to the contraction. When isoprenaline was added in this situation, the β-adrenoceptors were already occupied by the adrenaline so no inhibition was apparent. For the same mechanism to be applied in the seminal vesicle, it is necessary to postulate that noradrenaline and phenylephrine occupy the β-adrenoceptors. An alternative mechanism to account for the inability to detect the β-adrenoceptors in preparations contracted with sympathomimetic agents could involve the speed of the smooth muscle contraction. It could be suggested that acetylcholine-induced contractions have a fast phase that is inhibited by isoprenaline and a slow phase that is not, while noradrenaline caused only the slow contraction. Acetylcholine did produce a fast contraction, but not a slow phase. The contractions with noradrenaline were slow, but transmural stimulation resulted in a contraction at least as fast as that produced by acetylcholine The velocity of the contraction would not appear to be the reason for our inability to detect the inhibitory β-adrenoceptors in the seminal vesicle when using sympathomimetic agents to contract the tissue.

It was possible to demonstrate that high concentrations of isoprenaline increased the size of the contractions of the tissue to acetylcholine. This, together with the ability of very high concentrations of isoprenaline to cause a contraction, suggested that we were observing the weak α-adrenoceptor stimulating action of isoprenaline. This action on the α-adrenoceptors was confirmed by the use of pharmacological antagonists. It is worth noting that salbutamol did not increase the contractions to acetylcholine above the level of the control responses in any concentration used in these experiments. Thus, salbutamol is either free of an action on the α-adrenoceptor, or this action is so feeble as to be insignificant when compared with that of isoprenaline.

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