Brit. J. Pharmacol. (1965), **24**, 194–204.

SYMPATHETIC β-RECEPTORS AND THE GUINEA-PIG VAS DEFERENS

BY

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(*Received June* 4, 1964)

The innervation of the vas deferens judged on a pharmacological basis has been investigated by several authors. The transmission from postganglionic fibre to cell is believed to be adrenergic: vasa deferentia from guinea-pigs treated with reserpine give much smaller contractions to nerve stimulation than do normal tissues (Huković, 1961), while guanethidine and bretylium in low concentrations cause complete abolition of responses to either nerve stimulation or transmural stimulation (Bentley & Sabine, 1963). However, Boyd, Chang & Rand (1960) suggested that the effects of certain antiadrenaline agents on the guinea-pig vas deferens might be due to the anticholinesterase activity of these compounds, and the idea was elaborated by Burn & Weetman (1963) to produce further evidence of a " cholinergic link " at the end of the postganglionic fibre, whereby a nerve impulse liberates acetylcholine from a store and this mediates release of the adrenergic transmitter. In 1962, Sjöstrand had tentatively postulated the existence of ganglia between the point of stimulation of the nerve and the muscle, and subsequently Bentley & Sabine (1963) and Birmingham & Wilson (1963), all of whom compared transmural with nerve stimulation, showed more clearly that the hypogastric nerve was not made up simply of postganglionic fibres. Vogt (1963) demonstrated histologically that chromaffin tissue and ganglia occurred in the hypogastric nerve of the dog. Ferry (1963) provided evidence from the anaesthetized guinea-pig that ganglia were present in the nerve in close proximity to the vas deferens.

Despite the varied interpretations of the vas deferens innervation it seemed possible that, because of the undisputed adrenergic mechanism, both α- and β-receptors (Ahlquist, 1948) could be present in the vas deferens, and therefore the following investigation was undertaken.

METHODS

The preparation was made as described by Burn & Weetman (1963). Male albino guinea-pigs were used, weighing 350 to 450 g. The tissue was suspended in a 60-ml. overflow isolated organ-bath containing McEwen's (1956) solution at 32° C, through which passed a mixture of 95% oxygen and 5% carbon dioxide. The nerve was stimulated by rectangular pulses of 0.5 msec duration through electrodes like those used by Burn & Rand (1960). Supramaximal voltages and optimal frequencies of 15 or 20 shocks/sec were used. Each preparation was stimulated at 60-sec intervals, and a total of 60 shocks was delivered during each period of stimulation.

Cumulative log concentration/response curves were obtained by adding a compound to the bath 55 sec before a nerve stimulation, and at either 1- or 2-min intervals more of the compound was added (sufficient

to give logarithmically increasing concentrations). The response to a particular concentration was then expressed as a percentage change in height of the first or second contraction compared with the control height before the addition of any compound.

In some experiments the vas deferens was set up without the hypogastric nerve and contractions were obtained to transmural stimulation at a frequency of 20 shocks/sec and 90 V. The electrodes comprised two platinum wires each 2 cm long which were placed one on each side of the vas deferens at a distance of 2 mm (Birmingham & Wilson, 1963). Whenever contractions were induced by drugs the vas deferens without nerve was suspended in a 20-ml. organ-bath, and solutions were changed by draining the bath contents. Contractions were recorded on a smoked drum with an isotonic frontal-writing lever giving approximately a sevenfold magnification.

The drugs used were: adrenaline hydrochloride, acetylcholine chloride, dichloroisoprenaline, hexamethonium bromide, isoprenaline sulphate, noradrenaline bitartrate, phentolamine and pronethalol, and concentrations are expressed as g/ml. of the salts.

RESULTS

Actions of adrenaline and isoprenaline

Adrenaline. When regular contractions of the vas were obtained by nerve stimulation, the effect produced by adrenaline or isoprenaline depended on the concentration and the time for which the drug was in contact with the tissue. Concentrations of adrenaline greater than 1×10^{-6} g/ml. increased the size of contractions, equilibrium occurring when a plateau was reached after 3 to 10 min contact (five experiments). However, when concentrations of 1×10^{-9} to 1×10^{-6} g/ml. were used, an initial reduction in size of the response was observed, the effect usually being greatest within 60 sec of contact, that is after one contraction (six experiments). Fig. 1 illustrates this dual action of adrenaline; in two experiments the

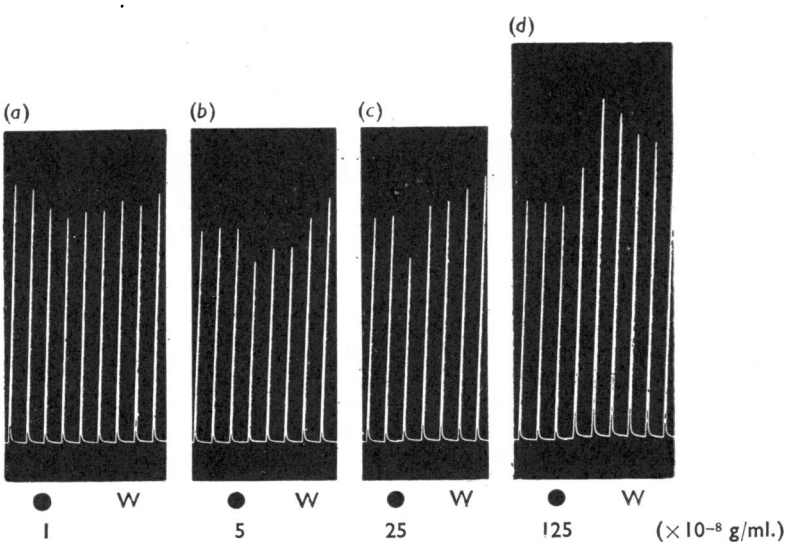

Fig. 1. Responses of guinea-pig isolated vas deferens preparation to hypogastric nerve stimulation when adrenaline was added, at the dots (concentrations in 10^{-8} g/ml.). The nerve was stimulated at 60-sec intervals with rectangular pulses of 0.5 msec duration and supramaximal voltage, at 20 shocks/sec, for 3 sec. In (*a*), (*b*) and (*c*) increasing concentrations of adrenaline produced greater, but more transient, reductions in response to nerve stimulation, and in (*d*) augmentation only of the responses occurred. At W the adrenaline was washed out.

maximum reduction occurred with the second contraction. At equilibrium with concentrations from 1 to 50×10^{-9} g/ml. the responses levelled at a height lower than the original contractions, but from 5×10^{-8} to 1×10^{-6} g/ml. the reduction was more transient and either complete recovery or even an increase above the original level ensued until the responses levelled within 2 to 5 min contact.

Isoprenaline. Although the effects with isoprenaline resembled those with adrenaline qualitatively, three points of difference were apparent. Firstly, the maximum reduction after a suitable concentration of isoprenaline usually occurred at the second (instead of the first) contraction. Secondly, in optimal concentrations (from 3 to 30×10^{-7} g/ml.) isoprenaline reduced the contractions approximately four- to six-times more than adrenaline. Thirdly, the concentration required for augmentation was 100- to 300-times greater than that for adrenaline. In view of these findings, cumulative log concentration/response curves were obtained with adrenaline and isoprenaline. Fig. 2 shows a tracing from an experiment where adrenaline and isoprenaline were added in this manner. In all, thirty-two experiments were performed.

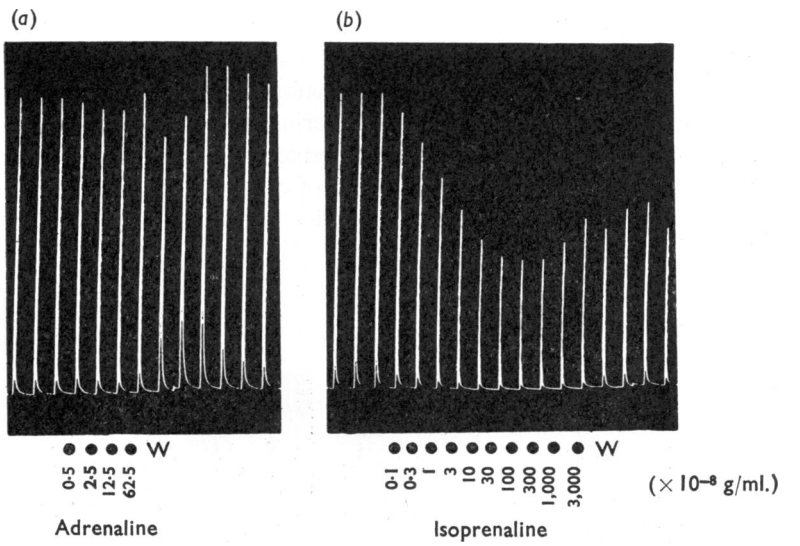

Fig. 2. Responses of guinea-pig vas deferens preparation to hypogastric nerve stimulation with rectangular pulses of 0.5 msec duration and supramaximal voltage, at 15 shocks/sec, for periods of 4 sec at 60-sec intervals. In (*a*), adrenaline hydrochloride added to bath at each dot, with an interval of 55 sec before the next contraction. Amounts were added so that the concentration ($\times10^{-8}$ g/ml.) indicated at each point was the total present in the bathing fluid. In (*b*), isoprenaline sulphate was added at similar intervals to the preparation 20 min after the adrenaline had been washed out. W indicates start of washing. This stepwise addition of the amines produced the cumulative log concentration/response curves referred to in the text.

Actions of sympathetic receptor blocking agents

Phentolamine. When the hypogastric nerve was stimulated at frequencies of 15 or 20 shocks/sec, concentrations of phentolamine from 1 to 10×10^{-7} g/ml. produced small reductions in the height of response. If adrenaline was then added to the preparation in the

presence of phentolamine, using a concentration which had previously increased the size of responses to nerve stimulation, the ensuing result was a reduction in height. Fig. 3 shows how the initial effect of adrenaline was modified by phentolamine. In four of five experiments the initial reduction of response with 5 to 10×10^{-7} g/ml. of isoprenaline was later increased in the presence of phentolamine. In two experiments the augmentation of the response to nerve stimulation produced by high concentrations of isoprenaline was abolished by the addition of 1×10^{-6} g/ml. of phentolamine.

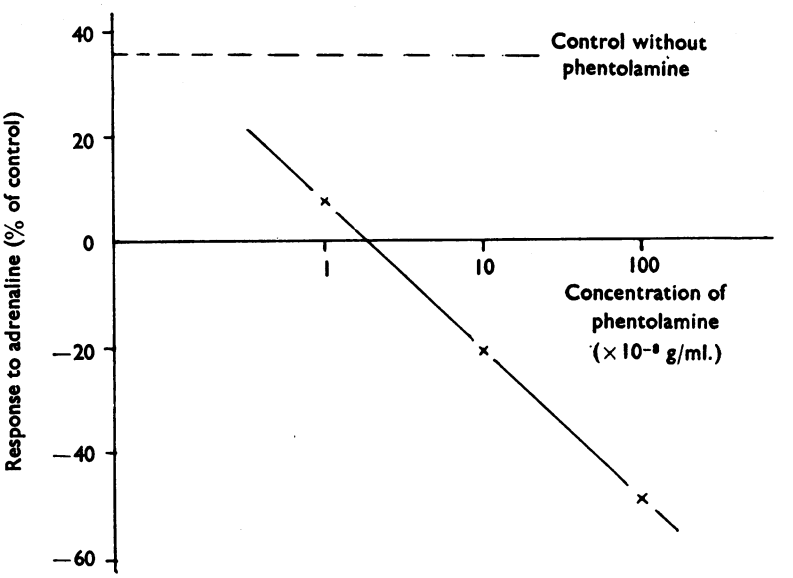

Fig. 3. Effect of adding 1.25×10^{-6} g/ml. of adrenaline to a guinea-pig vas deferens-hypogastric nerve preparation, stimulated as in Fig. 1. The initial augmentation of response was converted to a reduction in the presence of increasing concentrations of phentolamine (abscissa, log scale).

Dichloroisoprenaline and pronethalol. Addition of amounts from 1×10^{-8} to 1×10^{-6} g/ml. of either of these substances produced a slight increase in the height of contractions. When the concentration was increased to 1 or 10×10^{-5} g/ml. a reduction in size occurred which was sometimes preceded by a transient increase. A concentration of 1×10^{-6} g/ml. of dichloroisoprenaline (or pronethalol) was usually sufficient to abolish completely the reduction of responses seen with 1×10^{-7} g/ml. of isoprenaline or with 1×10^{-6} g/ml. of adrenaline in the presence of phentolamine.

A more detailed analysis was gained from observations on the modifications of the cumulative log concentration/response curves to isoprenaline. Control curves with isoprenaline showed progressive reductions in the size of responses as the concentration was increased until a maximum effect occurred with a concentration between 3 and 30×10^{-7} g/ml. Further increases in concentration caused gradual augmentation of contractions, so that, by plotting log concentration of isoprenaline against percentage reduction from the initial level, inverted parabolic curves were produced. Fig. 4 shows a tracing to illustrate how dichloroisoprenaline altered the nature of the response to isoprenaline. It will be seen

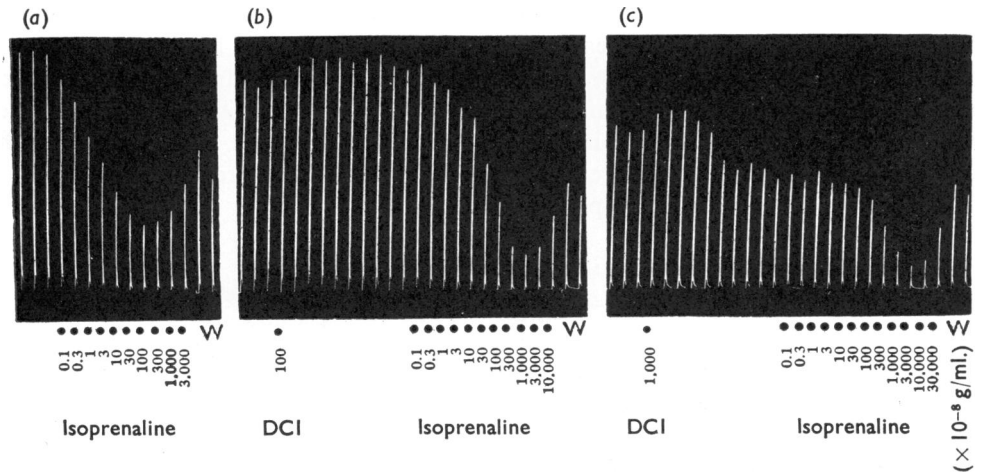

Fig. 4. Cumulative concentration/response relationships with isoprenaline (obtained as in Fig. 2, *a*). The hypogastric nerve was stimulated with rectangular pulses of 0.5 msec duration and supramaximal voltage, at 20 shocks/sec, applied for 3 sec every 60 sec. (*a*) Effect of isoprenaline alone; (*b*) the effect of isoprenaline in the presence of 1×10^{-6} g/ml. of dichloroisoprenaline (DCI), which initially itself slightly increased the responses to nerve stimulation; (*c*) the effect of isoprenaline after 1×10^{-5} g/ml. of dichloroisoprenaline, which in this higher concentration first increased and then reduced responses. All concentrations $\times 10^{-8}$ g/ml. W, wash.

that the optimum concentration of isoprenaline was higher in the presence of dichloroisoprenaline (1×10^{-5} g/ml. of isoprenaline produced maximal reduction with 1×10^{-6} g/ml. of dichloroisoprenaline present, and 3×10^{-5} g/ml. of isoprenaline was required with 1×10^{-5} g/ml. of dichloroisoprenaline). Dichloroisoprenaline itself at 1×10^{-6} g/ml. slightly augmented the response to nerve stimulation, whilst at 1×10^{-5} g/ml. there was an initial increase followed by reduction of responses. Observation of the graphs of log concentration of isoprenaline against percentage reduction of original response showed that the inverted parabola had been shifted *in toto* to the right. Furthermore, the maximal reduction in response to isoprenaline was greater in the presence of dichloroisoprenaline. The parallelism of the curves suggested a competitive antagonism between dichloroisoprenaline and isoprenaline, and experiments in which pronethalol was used instead of the former gave a similar result.

Combined effects of adrenaline, noradrenaline and isoprenaline

Cumulative log concentration/response curves to isoprenaline were obtained after the initial application of adrenaline (1 to 10×10^{-6} g/ml.), noradrenaline (1 to 10×10^{-6} g/ml.) or isoprenaline (1 to 10×10^{-5} g/ml.), which by themselves caused augmentation or only slight reduction. Under these circumstances there was a flattening or even disappearance of the parabolic shape of the control curve. Indeed, it was possible using carefully chosen concentrations of these amines to observe only increases in size of the responses when a cumulative addition of isoprenaline was undertaken. Fig. 5 illustrates such an experiment. Nine experiments were performed. A summary of the main effects is given in Table 1.

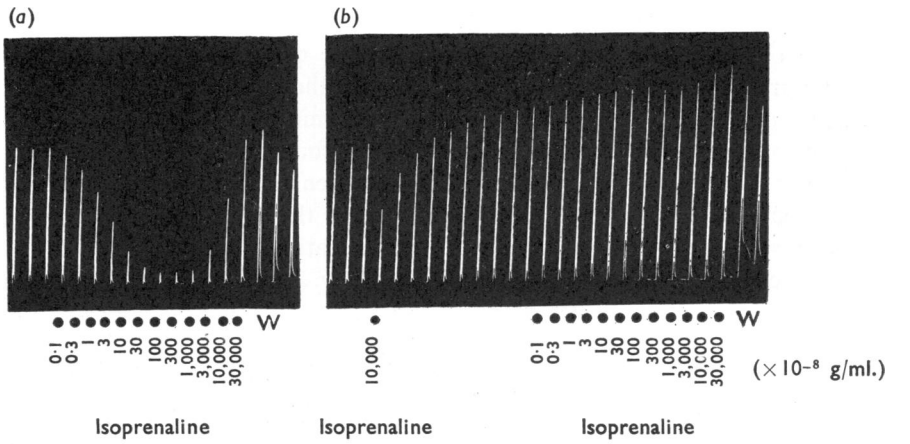

Fig. 5. Cumulative concentration/response relationships with isoprenaline. The hypogastric nerve was stimulated with rectangular pulses of 0.5 msec duration and supramaximal voltage, at 15 shocks/sec, applied for 4 sec every 60 sec. (*a*) The effect of increasing the concentration of isoprenaline at 60-sec intervals in a logarithmic progression. (*b*) The effect of a similar cumulative addition of isoprenaline when a concentration of 1×10^{-4} g/ml. of isoprenaline had already been present in the bath for 10 min. The addition of this single high concentration of isoprenaline had caused a transient reduction followed by augmentation of responses to nerve stimulation. All concentrations $\times 10^{-8}$ g/ml. W, wash.

TABLE 1

SUMMARY OF THE EFFECTS OF ISOPRENALINE, ADRENALINE, PHENTOLAMINE, PRONETHALOL AND DICHLOROISOPRENALINE ON THE GUINEA-PIG VAS DEFERENS PREPARATION STIMULATED TO CONTRACT EITHER BY ELECTRICAL EXCITATION OF THE HYPOGASTRIC NERVE OR BY ADDITION OF DRUGS

Type of stimulation	Drug	Concentration ($\times 10^{-6}$ g/ml.)	Effect on contractions of the vas	Antagonism
Electrical stimulation of nerve	Adrenaline	0·001–0·01	Reduction	
		0·01–1	Reduction followed by increase	
		>1	Increase	
	Isoprenaline	0·001–10	Reduction	
		10–100	Reduction followed by increase	
		>100	Increase	
	Phentolamine	0·1–1	Reduction	Reversed augmentation seen with high concentrations of adrenaline
	Dichloroisoprenaline or pronethalol	0·01–1	Increase	Antagonized the reduction produced by isoprenaline or by adrenaline in presence of phentolamine
		1–10	Reduction	
Acetylcholine	Isoprenaline	0·01–10	Reduction	
		>10	Increase	
Noradrenaline	Isoprenaline	0·01–10	Reduction	
		>10	Increase	
Adrenaline	Isoprenaline	<10	No effect	
		>10	Increase	

Direct effects on the vas deferens without nerve stimulation

Instead of eliciting responses by stimulating the hypogastric nerve electrically, acetylcholine, noradrenaline or adrenaline was added to the bath in concentrations high enough to cause a direct contraction of the muscle. A 3-min cycle with 60-sec contact time was adequate for regular responses with acetylcholine, but it was found necessary to increase the cycle to 4 or 5 min with adrenaline and noradrenaline. Isoprenaline was added to the bath 55 sec before these drugs in order to obtain the same time relationships as in the experiments with nerve stimulation. In concentrations from 1×10^{-8} to 1×10^{-5} g/ml., isoprenaline reduced the contractions produced by acetylcholine or noradrenaline by up

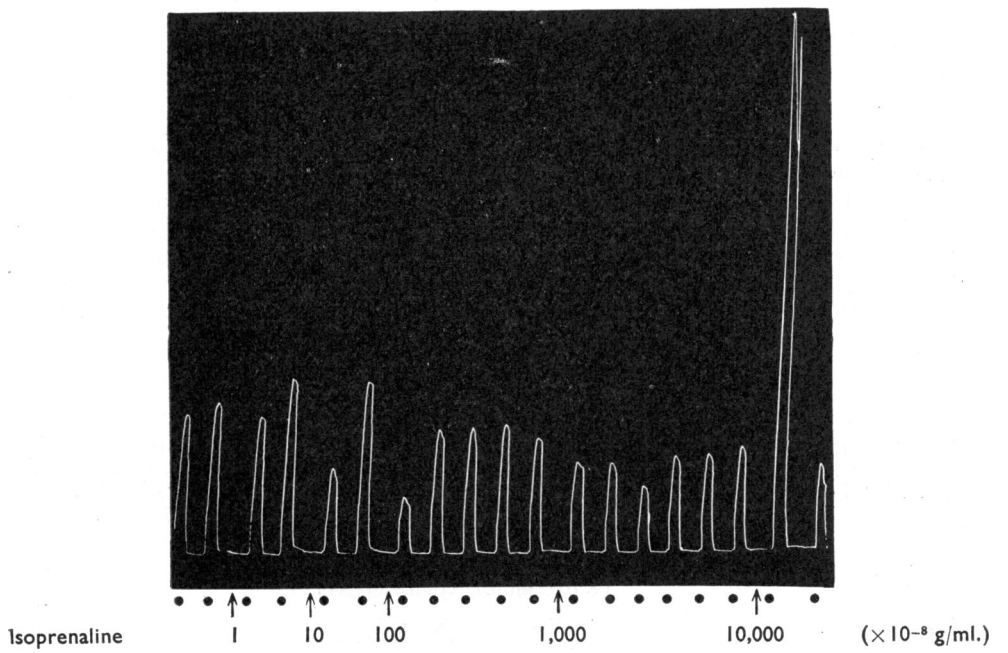

Fig. 6. Responses of guinea-pig vas deferens preparation to acetylcholine chloride at the dots in a concentration of 1×10^{-6} g/ml. for 60 sec and added on a 3-min cycle. At the arrows, isoprenaline was added in the concentrations ($\times 10^{-8}$ g/ml.) indicated 55 sec before the acetylcholine-induced contraction; then both were washed out after a total of 1 min 55 sec contact. A maximal reduction of the responses occurred with 1×10^{-6} g/ml. of isoprenaline; with 1×10^{-4} g/ml. the response to acetylcholine was greatly increased.

to 80% of the original height. When concentrations greater than 1×10^{-5} of isoprenaline were added to the bath, although no direct effect occurred, the responses to acetylcholine and noradrenaline became much greater. Fig. 6 shows the effect of isoprenaline on acetylcholine-induced contractions. When contractions were induced with noradrenaline (5×10^{-6} g/ml.), isoprenaline in concentrations from 1×10^{-8} to 1×10^{-6} caused graded reductions of the responses, culminating in complete abolition at 1×10^{-6} g/ml. A concentration of 1×10^{-4} g/ml. increased the response to noradrenaline. The addition of pronethalol in concentrations of 1 to 10×10^{-4} g/ml. abolished the responses both to acetylcholine

and to noradrenaline. The responses to adrenaline, unlike noradrenaline, were not reduced by isoprenaline in any concentration, though amounts of the order of 1×10^{-5} g/ml. augmented the response to adrenaline. Pronethalol (1×10^{-3} g/ml.) reduced but did not abolish completely the contractions due to adrenaline.

Responses of the vas to acetylcholine were unaffected by phentolamine but adrenaline- and noradrenaline-induced responses were abolished completely by 1×10^{-6} g/ml. of phentolamine.

Transmural stimulation of the vas deferens

Contractions similar in size to those seen with nerve stimulation were obtained. In order to show that responses were due to postganglionic nerve stimulation, hexamethonium was added to the bath (Bentley & Sabine, 1963) in some experiments. Whilst concentrations of 1 and 10×10^{-6} g/ml. of hexamethonium caused small transient reductions in size, 1 and 2.5×10^{-4} g/ml. only increased responses. Both in the absence, and in the presence, of hexamethonium, isoprenaline log concentration/response curves followed a similar pattern to those obtained with the nerve-stimulated preparation. Dichloroisoprenaline also shifted the curve to the right.

pA_2 determinations with dichloroisoprenaline and pronethalol

Although the log concentration/response curve to isoprenaline was shallow, it was possible to differentiate between effects due to twofold differences in concentration. Constant responses were obtained with the addition of a concentration of isoprenaline sufficient to produce a submaximal effect. Isoprenaline was added 55 sec before a contraction due to nerve stimulation and was washed out for a fixed period of 45 sec immediately after the second contraction. Since the reductions occurring with low concentrations of adrenaline and isoprenaline were partially time-dependent, and the maximal effect with isoprenaline was usually seen on the second contraction, this response was used to calculate the percentage reduction with isoprenaline. Recovery from the effects of isoprenaline occurred within about 5 min so that an 8-min cycle was used. When constant responses were attained, the antagonist was added to the bath at such a time that 5 min elapsed before the second contraction occurred in the presence of isoprenaline, that is 5 min contact time of antagonist was allowed before the response which was used as a criterion of isoprenaline activity. The concentration of agonist in this instance was double that used before. The drugs were washed out and, still maintaining an 8-min cycle, single concentrations of isoprenaline were added until the responses were back to the original size. Then a further concentration of antagonist was added and the procedure was repeated. pA_2 values were calculated by extrapolation from graphs showing change in percentage response against log concentration of antagonist. pA_2 values (means of seven experiments each) for dichloroisoprenaline and pronethalol on this preparation were 6.6 and 6.7 respectively; Van Rossum (1963) obtained, on tracheal muscle of calf, values of 4.9 and 5.8 for dichloroisoprenaline and pronethalol respectively.

DISCUSSION

One explanation for the findings described here may be based on the actions of the drugs on membrane potentials of the smooth muscle. Ohlin & Strömblad (1963) reported an antagonism between isoprenaline and various agents on the vas deferens, and concluded

that isoprenaline was exerting a stabilizing effect on the membrane like that seen with adrenaline on the taenia coli of guinea-pig (Bülbring, 1957). When adrenaline stimulates the muscularis mucosae of pig oesophagus (Burnstock, 1960), the contraction is associated with a membrane depolarization and initiation of spike activity. It is probable, therefore, that the augmentation seen in these experiments both with adrenaline and very high concentrations of isoprenaline occurs by a depolarization of the membrane and subsequent increase in frequency of spike discharge. The inhibitory actions of these drugs would then be associated with membrane stabilization and a decrease in spike discharge.

However, an alternative explanation may be based on the dual receptor theory of Ahlquist (1948). It is suggested that the reduction, with low concentrations of adrenaline, of responses to nerve stimulation is due to β-receptor stimulation. As the concentration is increased, the responses are increased because of a predominating α-receptor stimulation. A similar view can explain the actions of isoprenaline, except that concentrations of about 1×10^{-4} g/ml. are required before α-stimulation is sufficiently great to overcome the β-effects and consequently to cause augmentation of responses. The reversal by phentolamine of the increase due to high concentrations of adrenaline may be caused by a block of the α-effects to reveal the β-stimulant action of adrenaline. The total shifting to the inverted parabolic log concentration/response curve to isoprenaline in the presence of dichloroisoprenaline and pronethalol indicates a competitive blocking of the β-receptors.

Both dichloroisoprenaline and pronethalol, in concentrations of 1×10^{-5} g/ml. and higher, reduced the vas deferens contractions. This is probably a β-sympathomimetic action and it is of interest that Vanov (1963) showed that pronethalol caused inhibition of rat isolated uterus and of rabbit isolated intestinal segments.

If sufficient tone existed in the isolated vas deferens, isoprenaline might be expected to cause relaxation. Since, however, the tone is negligible, contractions were induced by addition of acetylcholine or noradrenaline, and an antagonism with isoprenaline could be demonstrated. On the other hand, it was not possible to observe any reduction of adrenaline-induced contractions. Possibly when adrenaline contracts the vas the concentration is of such a magnitude that there is maximum β-stimulation although α-effects predominate; consequently isoprenaline causes no reduction. The inhibitory action of isoprenaline cannot be due to α-receptor blockade because phentolamine completely abolished the contraction to adrenaline in a concentration which had no effect on acetylcholine-induced contractions.

On stimulation of the nerve to the vas presumably both α- and β-receptors are occupied and muscular contraction occurs because the α-stimulant effects of the transmitter predominate over the β-effects. The contraction is smaller for given intensity of nerve stimulation than it would be if β-receptors were absent. This is borne out by the observation that a β-blocker (dichloroisoprenaline or pronethalol) in a low concentration produces a small but significant increase in the size of the contractions. This cannot be due to α-stimulant activity since the effect still occurred in the presence of phentolamine.

Whatever may be the explanations for events in the excitation of vas deferens smooth muscle, the isolated preparation from the guinea-pig affords a useful pharmacological tool in the examination of β-receptor blocking agents.

Since my demonstration to the British Pharmacological Society in January 1964 of the estimation of β-receptor blocking activity using the vas deferens of the guinea-pig, Holman

& Jowett (1964) have published results of some actions of catechol amines on the same smooth muscle preparation. It is interesting that the conclusion reached was that the findings might be explained by the existence of both α- and β-receptors in the vas deferens.

SUMMARY

1. Isoprenaline reduced the responses of the vas deferens to nerve stimulation in concentrations from 1×10^{-9} to 5×10^{-5} g/ml., and increased them in higher concentrations.

2. Adrenaline also had a dual action, but the size of the reduction was less than that produced by isoprenaline, and the threshold for augmentation was about 1×10^{-6} g/ml.

3. In the presence of phentolamine, adrenaline reduced the responses in concentrations which normally increased them. The inhibitory actions of adrenaline and isoprenaline were antagonized by dichloroisoprenaline and pronethalol.

4. When cumulative log concentration/response curves were obtained with isoprenaline, a parallel shift of the curve to the right occurred in the presence of sympathetic β-receptor blocking agents.

5. Adrenaline and noradrenaline antagonized the inhibitory effect of isoprenaline, but then a depression of the log concentration/response curve resulted. A high concentration of isoprenaline similarly depressed the curve.

6. The suggestion is made that both sympathetic α- and β-receptors are present in the guinea-pig vas deferens. Both dichloroisoprenaline and pronethalol acted as competitive antagonists to isoprenaline, and pA_2 values for these antagonists have been determined. The usefulness of the preparation to estimate β-blocking activity is discussed.

I am indebted to Dr R. Wien for advice and encouragement given to me during the course of this work and to Mr R. V. Scott for skilled technical assistance.

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