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The response to transmural stimulation of isolated arterial strips and its modification by drugs

GAVIN PATERSON

Transmural electrical stimulation of isolated aortic strips of rabbits, induced contractions of the muscle which were blocked by concentrations of cocaine and lignocaine which augmented the response to noradrenaline. The adrenergic neurone blocking agents bretylium, guanethidine and bethanidine also blocked selectively the response to transmural stimulation. With bretylium and guanethidine the responses to noradrenaline were augmented. α-Receptor blockade with piperoxan, phentolamine, phenoxybenzamine and dihydrogenated ergot alkaloids also blocked the responses to transmural stimulation. Cocaine in low concentration and dexamphetamine augmented the responses to electrical stimulation. They also delayed onset of block with adrenergic neurone blocking drugs and reversed this when it was already established. Hexamethonium and pentolinium, in concentrations which in other tissues are known to cause complete blockade of ganglionic transmission, had no effect on electrical stimulation. It is concluded that the responses to electrical stimulation are the result of excitation of post-ganglionic adrenergic axons in the walls of the arterial preparation. Strips of common carotid, superior mesenteric, renal, pulmonary and common iliac arteries of the rabbit gave responses to electrical stimulation similar to those of the aorta.

STRIPS of arteries have for many years been used for the study of the physiological and pharmacological responses of vascular smooth muscle (see Furchgott, 1955; Bohr, 1964; Green & Boura, 1964). A nerve-muscle preparation has been described for the pulmonary artery of the rabbit (Bevan, 1962; Bevan & Su, 1964) but, apart from this, attempts to obtain an uncomplicated neurogenic response in isolated arterial preparations have been unsuccessful (Furchgott, 1952; Leonard, 1957) mainly because of the form and parameters of electrical stimulation which have been necessary for eliciting a contraction in these preparations. Where square wave impulses were used it was necessary to use pulse durations of 10 to 50 msec and 50 to 100 V to evoke contractions, but it was found that responses to other forms of stimulus were subsequently depressed (Leonard, 1957). This led to the use of low voltage alternating current (50 to 60 cycles/sec) for inducing reproducible responses from arterial strips (Furchgott, 1952; Leonard, 1957; Gillis & Yates, 1963; Ghosh & Roddie, 1964). The contraction induced in this way had two components: during the period of stimulation a rapid contraction occurred; on cessation of stimulation a second, slower contraction appeared from which the muscle recovered slowly. The first phase appeared to be the result of direct stimulation of the muscle, but the second phase was ascribed by Furchgott to the release of an adrenaline-like substance in the arterial wall, since dibenamine blocked this phase of the response. This concept was strengthened by the results of Gillis & Yates (1963) using piperoxan, bretylium or reserpine, all of which abolished the second phase.

The introduction of transmural stimulation of smooth muscle (Paton, 1955) extended the possibilities for neurogenic electrical stimulation of

From the Department of Pharmacology, University of London, King's College, Strand, London, W.C.2.

isolated tissues and it is with this form of stimulation that the present experiments are concerned.

When artery strips are excited by transmural stimuli of short pulse duration, reproducible responses can be obtained which on pharmacological analysis are free from a "direct" component of stimulation and are susceptible to modification by substances affecting post-ganglionic adrenergic nerve mechanisms.

Experimental

Rabbits, 1·5 to 3 kg, were killed by a blow to the back of the neck. The thorax and abdomen were opened and the entire descending aorta dissected into cold Krebs solution gassed with 95% oxygen and 5% carbon dioxide. The aorta was extended under tension between two small artery clips secured by lengths of cotton. This facilitated clearance of fatty and connective tissue and the subsequent dissection. An incision was made about 2 mm from the cardiac end of the vessel and a spiral strip cut measuring about 3 mm wide by about 5 cm long. The intact ring of the artery at the cardiac end was used to fix the preparation on a perspex rod mount. The other end of the strip was attached to an isotonic frontal lever, of load 4 g and magnification $\times 12$, writing on a smoked paper. The stimulating electrodes were also an integral part of the assembly on which the muscle strip was mounted (for details see Birmingham & Wilson, 1963; Paterson, 1965) and consisted of two parallel wires cemented vertically on two sides of a Perspex channel with the aortic strip suspended between the wires. The muscle was arranged so that it presented its flat surface to the electrodes and was immersed at 37° in 75 ml Krebs solution gassed with 95% oxygen and 5% carbon dioxide.

ELECTRICAL STIMULATION

Some preliminary experiments were necessary to establish the optimum parameters of stimulation. The findings of these are described more fully in Results but optimum conditions for electrical stimulation were found to be with square wave impulses of 300 μsec duration and 100 to 120 V at a frequency of 20 or 25/sec. Both Palmer H44 and Multitone stimulators gave satisfactory responses. The preparation was stimulated for 1 min every 40 min, timed by a cam-operated micro-switch device. Responses to drugs were usually interposed between periods of transmural stimulation and were timed to occur 20 min after the end of stimulation. After setting up the preparation the responses to drugs and transmural stimulation increased for up to 2 hr during which time the muscle slowly relaxed. Responses were then uniform for a further 6 to 8 hr. When strips from arteries other than the aorta were used they were prepared in the same way.

Drugs used were: piperoxan hydrochloride; phentolamine hydrochloride; phenoxybenzamine hydrochloride; lignocaine hydrochloride; cocaine hydrochloride; dihydroergotamine methanesulphonate; bretylium

tosylate; guanethidine hemisulphate; bethanidine sulphate; guanoxan sulphate; hexamethonium bromide; pentolinium tartrate; dexamphetamine sulphate; (−)-noradrenaline bitartrate; angiotensin. Concentrations are expressed as final bath concentration (g/ml) in terms of the base.

Results

ELECTRICAL STIMULATION. EFFECTS OF VARYING THE FREQUENCY ON THE RESPONSE OF THE ARTERIAL STRIP TO TRANSMURAL STIMULATION

With a pulse of 300 μsec duration and 100 V, aortic strips were stimulated for 30 sec at frequencies of 1, 2, 5, 10, 20 and 50/sec, with 20 min between trains of stimuli. The contraction with a frequency of 1/sec was less than 10% of that at 50/sec in one preparation and in two out of three preparations was less than 1%. It was found that the lowest frequency at which a consistently useful contraction was obtained was 20/sec. The contraction at a frequency of 50/sec was always larger than that at 20/sec, but the height of contraction at 50/sec declined in 2–3 hr and was often accompanied by changes in "tone" of the preparation, whereas at 20/sec, responses were uniform for upwards of 6 hr.

EFFECTS OF VARYING THE INTERVAL BETWEEN STIMULI

Aortic strips were stimulated for 15 sec every 10 min with pulses of 300 μsec and 100 V at a frequency of 20/sec. Reproducible contractions were obtained, but relaxation to resting tension was incomplete and a raised base-line response was produced. When stimulated for 30 sec every 20 min using the same stimulus parameters, the strip gave larger contractions, but relaxation was almost always complete between periods of stimulation. This was the form of stimulation chosen where responses to stimulant drugs such as noradrenaline were not recorded. In most experiments, however, responses to noradrenaline were interpolated between responses to transmural stimulation and in these circumstances the period of electrical excitation was 1 min in every 40 min. The concentration of the noradrenaline was adjusted to give a contraction similar in height to that of transmural stimulation.

EFFECTS OF LOCAL ANAESTHETIC DRUGS

The two drugs chosen were lignocaine and cocaine. These had been shown to have only about one-fifth of the muscle-depressant properties of procaine on the rabbit isolated aorta (Åström, 1964). Lignocaine caused block of responses to transmural stimulation ranging from 30% block with 5×10^{-6} to 100% block with 4×10^{-5}. With these concentrations the response to noradrenaline was potentiated (with 4×10^{-5} lignocaine, the contraction with 10^{-8} noradrenaline was nearly doubled). With 2×10^{-5} and 4×10^{-5} cocaine, 75% and 90% block respectively of transmural stimulation were obtained, while again the response to noradrenaline was enhanced. Cocaine 5×10^{-6} enhanced the responses both to transmural stimulation or added noradrenaline. Both enhancement

and blockade by the local anaesthetics were quickly reversed on washing the preparation.

EFFECTS OF SUBSTANCES BLOCKING α-RECEPTORS

Piperoxan (5×10^{-7} to 2×10^{-6}) caused partial to complete block of the responses to transmural stimulation and to noradrenaline. The effect of 10^{-6} piperoxan on transmural stimulation is illustrated in Fig. 1. The block reversed on washing.

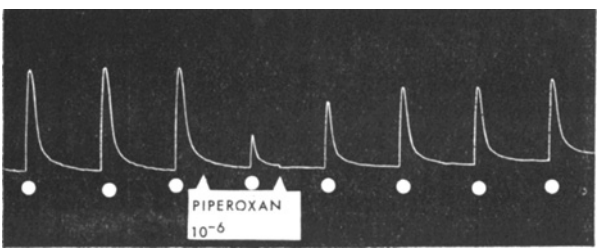

FIG. 1. Rabbit aortic strip. The blockade of responses to transmural stimulation by piperoxan. At the white spots the preparation was stimulated transmurally at a frequency of 20/sec for 30 sec every 20 min. The duration of the square wave pulse was 300 μsec and voltage was 120 V. Piperoxan (10^{-6} final bath concentration) was added to the bath and remained in contact with the muscle for the period indicated by the arrows. It was then washed out.

Phentolamine (10^{-7} to 10^{-6}) again blocked transmural stimulation and added noradrenaline, but, on washing, recovery was slower than with piperoxan (Fig. 2). Phentolamine (10^{-7}) did not alter the responses of the preparation to 2×10^{-9} angiotensin at a time when 2×10^{-9} noradrenaline was completely blocked and transmural stimulation was reduced to 10% of normal. Phenoxybenzamine (5×10^{-7} to 10^{-6}) blocked irreversibly the responses to both transmural stimulation and to

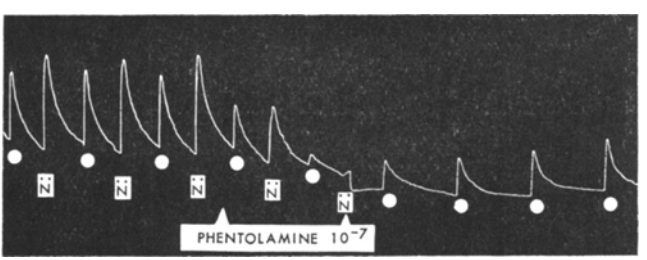

FIG. 2. Rabbit aortic strip. The blockade of responses to transmural stimulation and to noradrenaline by phentolamine. At the white spots the preparation was stimulated transmurally at a frequency of 20/sec for 1 min every 40 min. The duration of the square wave pulse was 300 μsec and voltage was 100 V. At N, noradrenaline (10^{-8}) was added to the bath and washed out after 4 min contact; the duration of contact with the muscle is indicated by the two dots. Phentolamine (10^{-7}) was added to the bath and remained in contact with the muscle for the period indicated by the arrows. It was then washed out. Concentrations are expressed as final bath concentration.

noradrenaline. Onset of block occurred in less than 10 min and despite several changes of Krebs over a 4 hr period, no recovery of adrenergic function was detectable. Again responses to angiotensin could still be elicited when both transmural stimulation and noradrenaline were completely blocked with 10^{-6} phenoxybenzamine.

With dihydroergotamine (DHE; 10^{-6}, 2×10^{-6}) and Hydergine (2×10^{-6}) block was complicated in four out of six experiments by a slowly developing contraction, but 70% block was obtained with 2×10^{-6} DHE and 50% with 2×10^{-6} Hydergine.

ADRENERGIC NEURONE BLOCKING AGENTS

Bretylium (5×10^{-7} to 2×10^{-6}) or guanethidine (2×10^{-7} to 10^{-6}) caused progressive blockade of transmural stimulation accompanied by a potentiation of the response to noradrenaline (Figs 3 and 4). On

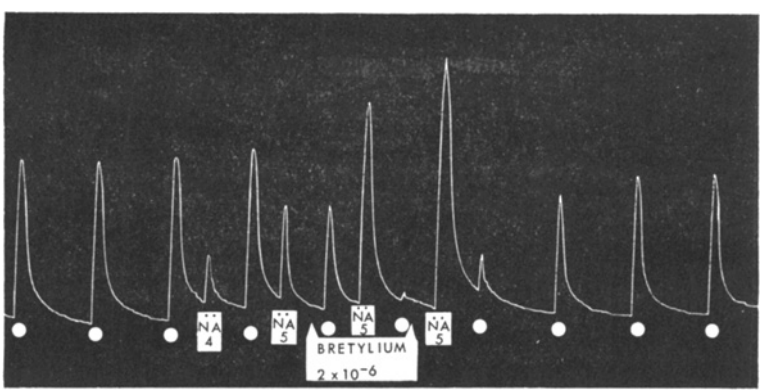

FIG. 3. Rabbit aortic strip. The blockade of responses to transmural stimulation, and enhancement of response to noradrenaline, by bretylium. At the white spots the preparation was stimulated transmurally at a frequency of 20/sec for 1 min every 40 min. The pulse width was 300 μsec and the voltage was 100 V. At NA4 and NA5, noradrenaline was added to the bath to give final concentrations of 4×10^{-9} and 5×10^{-9} respectively. The duration of contact of the noradrenaline with the muscle was 4 min and is indicated by the dots. Bretylium (2×10^{-6} final bath concentration) was added to the bath and remained in contact with the muscle for the period indicated by the arrows. It was then washed out.

changing the bathing fluid the block caused by bretylium was reversed in 1 hr, but that with guanethidine was unchanged or only slightly reversed after washing for 4 hr. With higher concentrations of bretylium or guanethidine (5×10^{-6}) complete block of transmural stimulation was accompanied by a slowly developing contraction which reversed on washing. Bethanidine (10^{-7} to 10^{-6}) caused block of transmural stimulation, but with the concentrations used no increase in the action of noradrenaline (Fig. 5).

Guanoxan (5×10^{-7} to 2×10^{-6}) caused a decrease in the effects of both transmural stimulation and noradrenaline. On washing, the response to noradrenaline recovered quickly, but transmural stimulation was still reduced 1½ hr after washing (Fig. 6).

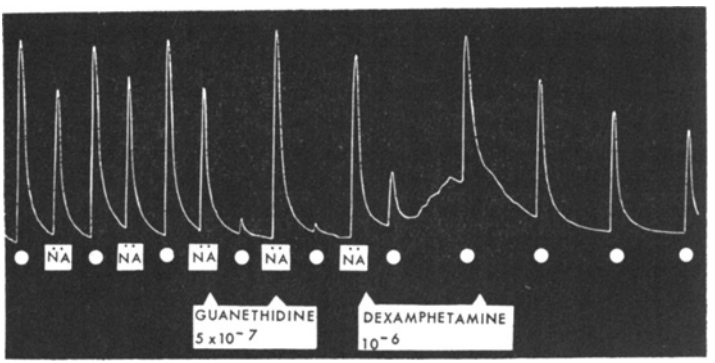

FIG. 4. Rabbit aortic strip. The blockade of responses to transmural stimulation, and enhancement of response to noradrenaline, by guanethidine. At the white spots the preparation was stimulated at a frequency of 20/sec for 1 min every 40 min. The pulse width was 300 μsec and the voltage was 100 V. At NA, noradrenaline (5×10^{-9}) was added to the bath and washed out after 4 min contact; the duration of contact with the muscle is indicated by the dots. Guanethidine (5×10^{-7}) was added to the bath and remained in contact with the muscle for the period indicated by the arrows. It was then washed out. Subsequently dexamphetamine (10^{-6}) was added to the bath for the period indicated by the arrows and was then washed out. Concentrations are expressed as final bath concentration.

REDUCTION OF ADRENERGIC NEURONE BLOCK BY DEXAMPHETAMINE AND BY COCAINE

Dexamphetamine (2×10^{-7} to 5×10^{-6}) reversed the sustained block of responses to transmural stimulation seen after washing out guanethidine (Fig. 4), bethanidine or guanoxan (Fig. 6). The reversal was usually accompanied by a slowly developing contraction of the aortic strip (Figs 4 and 6). On washing out, the reversal was only partly sustained (Fig. 4),

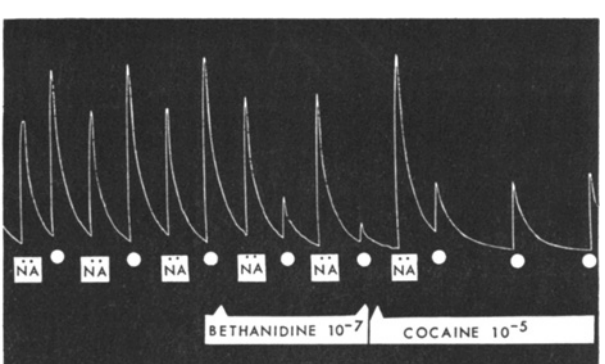

FIG. 5. Rabbit aortic strip. The blockade of responses to transmural stimulation by bethanidine. At the white spots the preparation was stimulated at a frequency of 20/sec for 1 min every 40 min. The pulse width was 300 μsec and the voltage was 100 V. At NA, noradrenaline (3×10^{-9}) was added to the bath and washed out after 4 min contact; the duration of contact with the muscle is indicated by the two dots. Bethanidine (10^{-7}) was added to the bath and remained in contact with the muscle for the period indicated by the arrows. It was then washed out. Cocaine was subsequently added to the bath at the arrow and left in the bath until the end of the experiment. Concentrations are expressed as final bath concentration.

but even when the dexamphetamine remained in the bath there was some reduction in the height of contraction (Fig. 6).

Three combinations of guanethidine and dexamphetamine (2×10^{-7} guanethidine and 2×10^{-7} dexamphetamine; 2×10^{-7} guanethidine and 4×10^{-7} dexamphetamine; 4×10^{-7} guanethidine and 4×10^{-7} dexamphetamine) were compared with the effect of 2×10^{-7} guanethidine alone, on responses to transmural stimulation (30 sec every 20 min) in four aortic strips from the same rabbit. Each combination was left in contact with one strip and then washed out. In the strip subjected to guanethidine only, full action developed in 30 min and was not reversed on washing. In all three preparations which had dexamphetamine added, responses to transmural stimulation were unchanged or slightly enhanced until the drugs were washed out; thereafter block of the responses developed in all three strips, but in none was it as complete as in the strip which had guanethidine only.

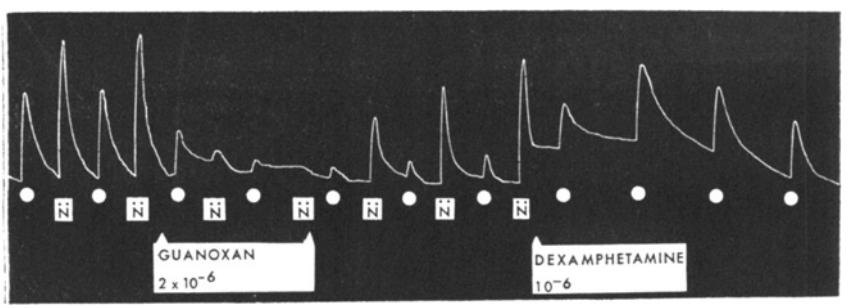

FIG. 6. Rabbit aortic strip. The blockade of responses to transmural stimulation and noradrenaline by guanoxan. At the white spots the preparation was stimulated transmurally at a frequency of 20/sec for 1 min every 40 min. The stimulus was 200 μsec and 100 V. At N, noradrenaline (5×10^{-9}) was added to the bath and washed out after 4 min contact; duration of contact is indicated by the two black dots. Guanoxan (2×10^{-6}) was added to the bath and remained in contact with the muscle for the period indicated by the arrows. Dexamphetamine (10^{-6}) was added to the bath at the arrow and remained in the bath for the remainder of the experiment. Concentrations are expressed as final bath concentrations.

Cocaine (5×10^{-6} to 10^{-5}) similarly reversed the persistent block caused by guanethidine (4×10^{-7}) and when given with the guanethidine prevented the onset of block until the drugs were washed out. Cocaine (5×10^{-6}) or dexamphetamine ($2{\cdot}5 \times 10^{-7}$) when given alone, increased the responses to transmural stimulation.

GANGLION BLOCKING AGENTS

Concentrations of hexamethonium and pentolinium up to 5×10^{-5} had no effect on transmural stimulation of aortic strips.

STRIPS FROM ARTERIES OTHER THAN THE AORTA

Arterial strips have been prepared from segments of the pulmonary, common carotid, renal, superior mesenteric and common iliac arteries of the rabbit and have yielded suitable responses on transmural stimulation.

These responses have not been fully investigated, but have not differed from those of the aorta to adrenergic neurone blockade. Strips from pulmonary arteries relaxed much more quickly after stimulation than did aortic strips.

ARTERIAL STRIPS FROM OTHER SPECIES

Aortic strips from guinea-pigs and carotid artery strips from cats also gave responses to transmural stimulation, but these have not been fully investigated.

Discussion

Isolated arterial muscle strips were stimulated transmurally with pulses of short duration if the strips were mounted between parallel wire electrodes. The mechanism by which responses to transmural stimulation were induced may be inferred from a consideration of the following.

The pulse width which gave optimal response of the tissue, 200 to 300 μsec, has been generally found, although not exclusively so, to stimulate nervous structures only. The local anaesthetic drugs, lignocaine and cocaine, in concentrations which did not block the action of noradrenaline on these preparations, abolished transmural stimulation. Substances which blocked the actions of noradrenaline on α-receptors in these strips also blocked concomitantly the responses to transmural stimulation; this was found with phentolamine, piperoxan, phenoxybenzamine, dihydroergotamine or Hydergine. The adrenergic neurone blocking agents, bretylium, guanethidine and bethanidine had a selective blocking effect on transmural stimulation. Also, cocaine or dexamphetamine potentiated the responses both to noradrenaline and transmural stimulation, and finally, the ganglion blocking agents hexamethonium and pentolinium had no effect on transmural stimulation.

On these counts the mechanism of the transmural stimulation was considered to be neurogenic and probably sited at post-ganglionic adrenergic nerve elements. One of the strongest pieces of evidence for this was the selective blockade with bretylium, guanethidine or bethanidine. These drugs have been shown to have selective blocking effects at adrenergic nerve endings only (Boura & Green, 1959, 1963; Maxwell, Plummer, Schneider, Povalski & Daniel, 1960).

The identity of the transmitter involved cannot be ascertained from the present experiments, but the catecholamine present in the walls of blood vessels is almost entirely noradrenaline and is associated with neural networks (Schmiterlöw, 1948; Falck, 1962; Norberg & Hamberger, 1964), so that it seems likely that this amine will be primarily concerned in transmural stimulation.

Potentiation of the actions of noradrenaline were seen with lignocaine, cocaine, bretylium or guanethidine. It seemed likely that these substances inhibited the uptake of noradrenaline into catecholamine stores. Muscholl (1961) has shown this to be so for cocaine, and Iversen (1965) confirmed this action and extended the observation to a number of other substances including bretylium and guanethidine. Bretylium and guanethidine also had a contractile action on aortic strips, but this was seen here to occur

with concentrations several times greater than those which caused block of transmural stimulation and potentiation of the action of noradrenaline. Kirpekar & Furchgott (1964) found that this contractile action of bretylium was due to release of noradrenaline from stores in the tissue since reserpinised strips did not respond to bretylium unless previously incubated with noradrenaline. Maxwell, Daniel, Sheppard & Zimmerman (1962) came to a similar conclusion for the action of guanethidine. Guanethidine is also known to deplete tissue stores of noradrenaline, an action which was found to be unrelated to adrenergic neurone block (Cass & Spriggs, 1961).

Reversal by dexamphetamine of the block of transmural stimulation caused by guanethidine, guanoxan and bethanidine was particularly striking where the block persisted after washing out these drugs. Although dexamphetamine has been shown here to have a potentiating action of its own, this would not in itself be sufficient to explain the reversal seen here, or the reduction in the action of guanethidine when the two drugs were administered together. These findings support the hypothesis that to some extent competition for a common site between dexamphetamine and guanethidine may explain this action (Day, 1962; Day & Rand, 1963).

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