

INTRAMURAL NERVES IN THE VENTRICULAR MYOCARDIUM OF THE DOMESTIC FOWL AND OTHER ANIMALS

BY

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Some of the first investigators to describe responses which can now be attributed to excitation of intramural cardiac nerve terminations were Bowditch (1871) and Ranvier (1880), both of whom worked on frog heart muscle, and Foster (1872) and Foster & Dew-Smith (1875) who worked on the snail heart. Gaskell (1883) observed that when Faradic shocks were applied to the atrium of the tortoise in a strength insufficient to stimulate cardiac muscle directly, a diminution and slowing of the beat was produced which, like the response to vagal stimulation, was mimicked by muscarine and prevented by atropine. Gaskell considered but rejected the possibility that Faradic stimulation excited vagal nerve fibres.

The possibility that electrical stimuli applied to cardiac tissue can excite nerve fibres within it, has been re-appreciated only recently (see Blinks & Koch-Weser, 1963). This paper describes responses produced by field stimulation applied to a strip cut from the wall of the right ventricle of the chick heart. The results are compared with those obtained with similar preparations from the pigeon, rat, and guinea-pig.

The work described in this paper formed part of a thesis approved for the degree of Doctor of Philosophy in the University of London. Brief preliminary reports of some of the results have already been published (Bolton & Raper, 1966).

METHODS

Preparations were taken from Silver Link chicks aged between 3 weeks and 3 months and killed by dislocating their necks. Preparations were also obtained from older chickens, adult fowls, adult pigeons, and young and adult guinea-pigs. A triangular piece of tissue was cut from the wall of the right ventricle. The base of this triangle coincided with the atrioventricular groove and the apex lay close to the apex of the heart. The base of the excised tissue was anchored in a 50 ml. organ bath by impaling it on three platinum hook electrodes. The apex was attached to a transducer for isometric tension recording. The transducer consisted of the valve RCA 5734 mounted in a housing similar to that described by Talbot, Lillenthal, Beser & Reynolds (1951). The output from the valve was led into a Sefram "Unitron" amplifier (Type 72A) and displayed on a Sefram "Rapidgraph" ink-writing pen recorder (Type R.P.5). An initial tension of 1 to 3 g was applied to the tissue.

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The bathing solution had the following composition (g/l.): NaCl, 6.92; KCl, 0.34; CaCl₂, 0.30; MgCl₂, 0.11; KH₂PO₄, 0.16; NaHCO₃, 2.1; glucose, 2.0; sucrose, 4.5. The solution was vigorously gassed with a mixture of 5% carbon dioxide and 95% oxygen and was maintained at 41 to 42° C for avian and 37 to 38° C for mammalian tissues. The bath was washed by overflow. Stimuli were applied between the outer hook electrodes or between platinum plates (7 mm square) placed at either side of and parallel to the tissue. Drug additions, electrical stimulation, and washout were performed automatically if desired.

The reagent 3-acetoxy-1-benzyl-1-methyl pyrrolidinium bromide (AHR-602) was supplied by A. H. Robins Co. Inc. and 4-(*m*-chlorophenylcarbamoyloxy)-2-butyryl trimethylammonium chloride (McN-A-343) was supplied by McNeil Labs Inc.

The concentrations and doses of noradrenaline refer to the base; those of other drugs refer to the salts. Reserpine was dissolved in 25% w/v ascorbic acid.

RESULTS

Chick ventricular strips

Electrical stimuli of less than 0.5 V were usually sufficient to drive strips when a 5 msec pulse duration was employed, but occasional preparations showed a higher threshold. In most preparations, varying the pulse duration from 0.5 to 50 msec had only a small effect on the threshold stimulus strength. Throughout the studies to be described, strips were continuously driven at constant rate (4 to 6/sec) using just suprathreshold stimuli of 0.5 to 5 msec duration applied between the hook electrodes anchoring the strip, except that just suprathreshold stimulation was replaced by field stimulation for short periods.

Drugs

Acetylcholine (10 ng/ml. or more) produced a depression of the force of contraction; concentrations of 100 to 200 ng/ml. depressed the force of contraction by 60 to 70%.

The ganglion stimulants tetramethylammonium (TMA), 4-(*m*-chlorophenyl carbamoyloxy)-2-butyryl-trimethylammonium (McN-A-343), or 3-acetoxy-1-benzyl-1-methyl pyrrolidinium (AHR-602) depressed the force of contraction. These depressions were not affected by hexamethonium or pempidine but were blocked by atropine or hyoscine. 1,1-Dimethyl-4-phenyl piperazinium (DMPP) or nicotine had a positive inotropic action as did acetylcholine or 3-acetoxy-1-benzyl-1-methyl pyrrolidinium in the presence of atropine.

Noradrenaline, adrenaline, dopamine or tyramine increased the force of contraction of ventricular strips.

Field stimulation

The field stimuli used were of greater strength but of the same duration and rate as the just suprathreshold stimuli used to drive the strip at constant rate (4 to 6/sec). They were applied either to the outer hook electrodes, normally used to drive the preparation, or to the plate electrodes set up on either side of, and parallel to, the tissue. It was found that a 5 msec pulse duration was more effective than 1 or 0.5 msec pulse durations in eliciting responses to field stimulation.

In a few preparations in which a 20 msec pulse duration was used, high field stimulus strengths caused an increase in tension resembling a contracture. Individual contractions of the strip were superimposed on this (Fig. 1). In the same preparations stimulation at

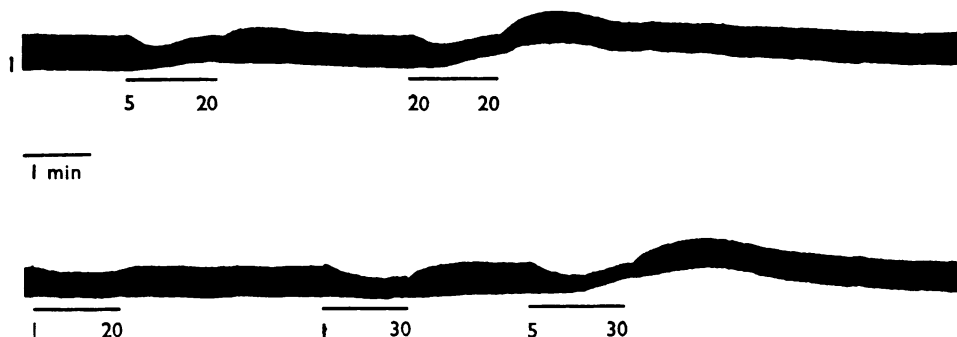


Fig. 1. Contractures occurring during field stimulation of a chick ventricular strip. The vertical line represents 1 g. Field stimulation was applied for the periods indicated by the horizontal lines. The left hand figure under each horizontal line is the pulse duration of the applied field stimulation in msec; the right hand figure under each horizontal line is the stimulus voltage of the applied field stimulation. Upper and lower records are continuous.

the same field stimulus strength but with a 5 msec pulse duration produced smaller contractures, but a 1 msec duration was not observed to do so (Fig. 1).

Positive inotropic responses to field stimulation

The results described under this heading were all obtained on preparations in the presence of atropine or hyoscine (0.5 to 1 $\mu\text{g/ml.}$) and driven at constant rate (4 to 6/sec). The addition of atropine or hyoscine (0.1 to 1 $\mu\text{g/ml.}$) produced little change in the force of contraction of most preparations (Fig. 5). However, in the presence of atropine or hyoscine, field stimulation caused an increase in the force of contraction of preparations. After field stimulation had ended the force of contraction gradually declined to the control value.

The α -receptor blocking drugs ergotamine (1 $\mu\text{g/ml.}$), dihydroergotamine (2 $\mu\text{g/ml.}$), tolazoline (5 $\mu\text{g/ml.}$), phentolamine (5 $\mu\text{g/ml.}$), and phenoxybenzamine (5 $\mu\text{g/ml.}$) were without effect or potentiated the positive inotropic responses to field stimulation, whereas, the β -receptor blocking drugs dichloroisoprenaline (0.2 to 1 $\mu\text{g/ml.}$), propranolol (0.1 to 0.2 $\mu\text{g/ml.}$) and pronethalol (0.2 to 1 $\mu\text{g/ml.}$) blocked them (Fig. 2). Methoxamine (15 $\mu\text{g/ml.}$) and isopropylmethoxamine (15 $\mu\text{g/ml.}$) reduced or abolished the positive inotropic responses to field stimulation only in concentrations which depressed the control force of contraction.

Adrenergic neurone blocking drugs in the following concentrations blocked the positive inotropic responses to field stimulation within 15 to 30 min following their addition: guanethidine 0.5 to 5 $\mu\text{g/ml.}$, guanoclor 0.5 to 2 $\mu\text{g/ml.}$, guanoxan 1 to 2 $\mu\text{g/ml.}$, bretylium 4 to 20 $\mu\text{g/ml.}$, bethanidine 4 to 20 $\mu\text{g/ml.}$, choline-2,6-xylyl ether 8 to 30 $\mu\text{g/ml.}$ Reversal of the blockade was attempted in most preparations. Dexamphetamine was added when blockade was maximal, without washing out the blocking drug. Small doses (0.5 $\mu\text{g/ml.}$) were added at first and left for at least 10 min. If no reversal had occurred the concentration was increased; reversal was infrequently observed and when it occurred only partial return of the response was found.

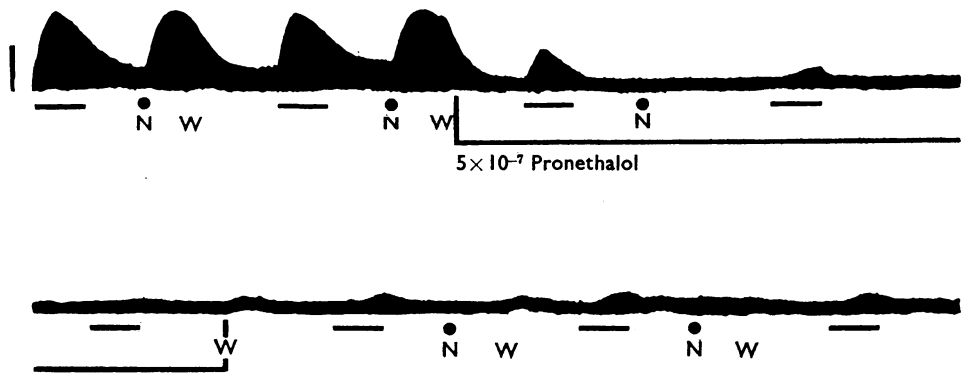


Fig. 2. The action of pronethalol on the responses of a chick ventricular strip preparation to 5 ng/ml. noradrenaline (N) and to field stimulation. Hyoscine ($0.5 \mu\text{g/ml.}$) was present throughout. The vertical line represents 1 g. Field stimulation was applied for 90 sec periods as indicated by the horizontal lines. The tissue was washed (w) as shown.

Concentrations of adrenergic neurone blocking drugs which produced blockade usually had some positive inotropic action (Fig. 3). This was especially marked with guanoclor. Guanoclor potentiated the response to field stimulation before producing blockade (Fig. 3A). DMPP produced a similar blockade of the response to field stimulation in this preparation confirming that it possesses an action resembling that of adrenergic neurone blocking drugs.

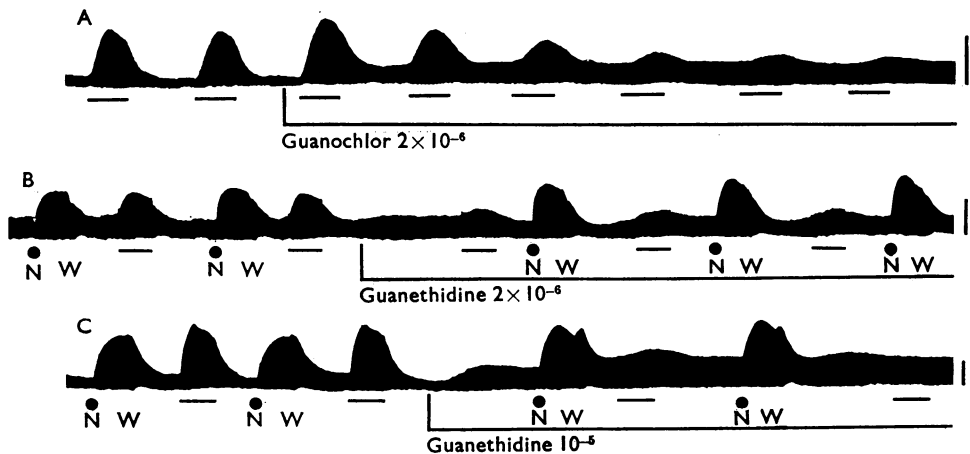


Fig. 3. The actions of adrenergic neurone blocking drugs on the responses of chick ventricular strips to noradrenaline and to field stimulation. Hyoscine ($1 \mu\text{g/ml.}$) was present throughout each experiment. The vertical lines represent 1 g in each record. Field stimulation was applied for 90 sec periods as indicated by the horizontal lines. The tissues were washed (w) as shown. Action of guanoclor, A, action of guanethidine, B, and C. 20 ng/ml. noradrenaline was added at N in B, in C the concentration of noradrenaline was 10 ng/ml.

In some experiments field stimulation and noradrenaline were applied alternately to the tissue. The concentrations of noradrenaline were such as to produce positive inotropic effects similar in size to those evoked by field stimulation. Guanethidine blocked the effects of field stimulation, while it potentiated the responses to added noradrenaline (Fig. 3B and C).

The following concentrations of local anaesthetics were needed to depress the positive inotropic responses to field stimulation by approximately 50%: cinchocaine 1 $\mu\text{g/ml.}$, cocaine 40 $\mu\text{g/ml.}$, lignocaine 40 $\mu\text{g/ml.}$, procaine 100 $\mu\text{g/ml.}$ These concentrations also depressed the control force of contraction and irregularities of beating occurred. There was little evidence that local anaesthetics were acting selectively on nervous elements. The feeble action of procaine is notable as it was later found to be much more potent in blocking the inhibitory effects of field stimulation.

A batch of day-old chicks was reared and when 6 weeks old divided into two groups, one group being given 3 mg/kg reserpine each day for 3 days while the other group was treated with the vehicle solution (25% w/v ascorbic acid). Ventricular strips from chicks of each group were used alternately under identical conditions and field stimulation was applied in the presence of atropine. The relationship between the stimulus strength and the maximum force of contraction attained during field stimulation was examined in 5 chicks from each group, using 2 stimulus pulse durations. The basal force of contraction was taken as the average of all control readings just before each occasion on which field stimulation was applied. The results are shown in Fig. 4.

The average basal force of contraction of reserpinized strips was about 2.5 times that of the controls. This difference was statistically significant ($P < 0.002$). In the reserpinized group, field stimulation produced further increases of about 50% in the force of contraction, despite the fact that the average force of contraction of the reserpinized strips was already roughly equal to the average maximum force of contraction obtained in the controls during field stimulation. In reserpinized preparations, as in the controls, a stimulus pulse duration of 5 msec was more effective than one of 0.5 msec in producing a positive inotropic effect. The increase in the force of contraction of reserpinized strips produced by field stimulation was abolished by β -receptor blocking drugs and by adrenergic neurone blocking drugs in concentrations lower than those needed to block the positive inotropic effects of field stimulation in the controls.

The ganglion blocking agents pempidine (150 $\mu\text{g/ml.}$), mecamylamine (20 $\mu\text{g/ml.}$), and hexamethonium (1 mg/ml.) were without appreciable effect on the increase in the force of contraction resulting from field stimulation. Cocaine (20 ng/ml. to 5 $\mu\text{g/ml.}$) was not observed to potentiate the responses to field stimulation or noradrenaline, although a slight increase in the control force of contraction occurred in some experiments.

Inhibitory responses to field stimulation

The results described under this heading were all obtained on preparations which were first treated with 20 $\mu\text{g/ml.}$ guanethidine to block the positive inotropic effects to field stimulation. In these preparations field stimulation caused a depression of the force of contraction. When field stimulation ended the force of contraction then increased rapidly to greater than the control level before field stimulation. Following this the force of

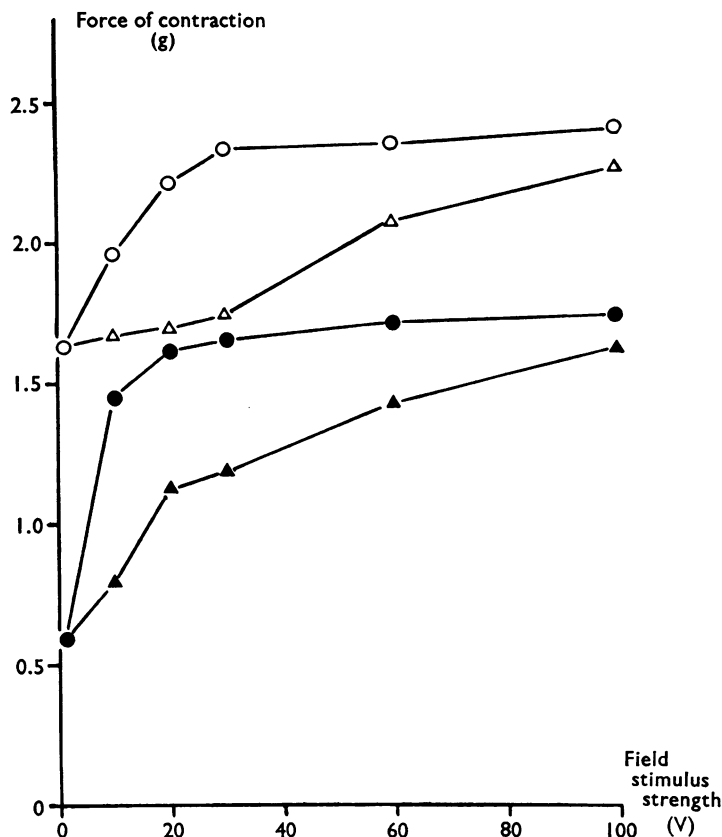


Fig. 4. The effects of reserpine pretreatment on the relationship between the field stimulus strength and the maximum increase in the force of contraction attained during field stimulation. All results were obtained in the presence of $1 \mu\text{g/ml}$ atropine. The closed symbols are results obtained on 5 control strips and the open symbols are the results obtained on 5 strips pretreated with 3 mg/kg reserpine daily for 3 days. Field stimulation consisted of pulses of either 5 msec (circles) or 0.5 msec (triangles) duration.

contraction became less than during the control period and further oscillations occurred lasting up to 6 min after the period of field stimulation. Field stimulation caused depressions of the force of contraction in many preparations which had not been previously treated with guanethidine. Field stimulation also caused depressions of the force of contraction in strips completely free of atrial tissue which were impaled by their apical ends on the hook electrodes, suggesting that the inhibitory nerves extended to the apical regions and were not confined to the basal portions of the ventricles as has been suggested to be the case in mammals (Keele & Neil, 1961). Reversing the polarity of the field stimulation did not change the response.

Depressions were obtained with field stimulation in strips from chicks up to 6 months old or from adult fowls. Although the physical dimensions of the strips from fowls older than about 4 months were greater than those of strips from younger fowls, the force of

contraction of preparations from younger fowls was much greater and the mode of beating more regular. Field stimulation in preparations from fowls older than about 4 months produced poor depressions. When the force of contraction was augmented with noradrenaline (20 to 100 ng/ml.), the regularity of beating improved and the depressions obtained with field stimulation or with acetylcholine were potentiated.

Figure 5A shows the marked reduction of the inhibitory response to field stimulation by 100 ng/ml. atropine. Maximal blockade was obtained 5 to 6 min after the addition of atropine and the blockade was slowly reversed by washing. In some preparations larger

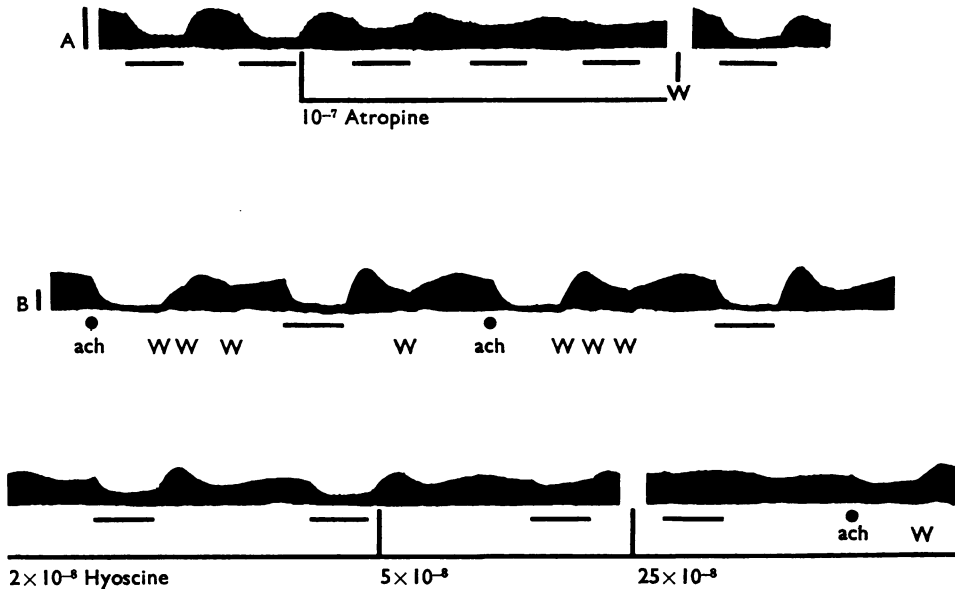


Fig. 5. A, the effects of atropine and B, the effects of hyoscine on the responses of chick ventricular strips to 0.2 μ g/ml. acetylcholine (ach) and to field stimulation. Upper and lower records in B are continuous. The vertical lines represent 1 g. Preparations were subjected to field stimulation for 2 min periods as indicated by the horizontal lines. The tissue was washed (w) as indicated. Both preparations had been previously treated with guanethidine (20 μ g/ml.) for 15 min.

concentrations of atropine or hyoscine were required to produce blockade. In the experiment illustrated by Fig. 5B, even allowing 5 min for each dose of hyoscine to act, complete blockade was not produced until 0.25 μ g/ml. was present. Although the inhibitory response to field stimulation was abolished, this concentration of hyoscine did not abolish the response to 0.2 μ g/ml. acetylcholine, a concentration of acetylcholine which, before the addition of hyoscine, produced depressions equal in size to those obtained with field stimulation.

Physostigmine (30 ng/ml. to 1 μ g/ml.) and edrophonium (0.5 to 3 μ g/ml.) were used in attempts to potentiate the depressions obtained with field stimulation. The above concentrations of anticholinesterases produced a slowly developing reduction in the control force of contraction. If the depression produced by field stimulation were

potentiated after the addition of an anticholinesterase, the force of contraction did not return to the control level unless the anticholinesterase was washed from the bath, and then only slowly. It was not possible in any preparation to potentiate the response to field stimulation with a concentration of anticholinesterase which did not at the same time depress the control force of contraction.

Cocaine (10 $\mu\text{g/ml.}$) had no effect on the inhibitory responses to field stimulation while lignocaine (10 $\mu\text{g/ml.}$) potentiated the depressions obtained. De Elío (1948) observed that cinchocaine potentiated the action of acetylcholine on rabbit atria. Procaine (10 to 20 $\mu\text{g/ml.}$) reduced or abolished the depressions obtained with field stimulation.

As procaine was relatively potent in blocking the inhibitory effect of field stimulation, compared with the other local anaesthetics used and compared with its effects on the positive inotropic effect of field stimulation, its selective action on the inhibitory response to field stimulation was examined in more detail. Doses of acetylcholine were added to the bath to produce depressions about equal to depressions produced by field stimulation. Procaine was found to be at least as potent in blocking the inhibitory effects of field stimulation as it was in blocking the responses to added acetylcholine, and in some cases it was more potent. Hyoscine was also more potent in blocking the inhibitory effects of field stimulation than in blocking responses to acetylcholine (Fig. 5B).

The actions of triethylcholine (TEC) and tetraethylammonium (TEA) both on the maximal depression of the force of contraction and on its maintenance during field stimulation were examined. Field stimulation at a frequency of 4 to 6 impulses/sec was applied for 5 min in every 10 min. Figure 6 illustrates the actions of TEC; TEA produced similar effects. In the control responses the maximum depression of the force of contraction was maintained for the whole period of field stimulation. Five minutes after the addition of TEC (0.25 mg/ml.) the control force of contraction was usually increased and it continued to increase for 15 to 20 min after its addition. In about half of the preparations, field stimulation, applied 5 min after the addition of triethylcholine, produced a greater decrease in the force of contraction than that produced in the absence of TEC. The contour of the first response obtained after the addition of TEC differed markedly from the controls; after the maximum depression had occurred the force of contraction then increased towards the control level despite the continuing field stimulation. This effect became progressively more marked in subsequent responses and was maximal about 30 minutes after the addition of TEC. The maximum depression obtained was also progressively reduced, the reduction being maximal 30 min after TEC. The response obtained 35 min after TEC addition (Fig. 6) showed that field stimulation exerted no effect on the force of contraction after about 3 min of continued stimulation. Control preparations stimulated for 5 min periods in every 10 min produced responses in which the maximal depression was maintained throughout the period of field stimulation (see control responses in Fig. 6).

After the addition of choline (50 $\mu\text{g/ml.}$) in the continuing presence of TEC, not only was the maximal inhibitory response much increased but it was then maintained for the whole period of field stimulation (Fig. 6). The effects of TEA were less well reversed by choline; Bowman, Hemsworth & Rand (1962) made similar observations in the cat sciatic nerve-tibialis anterior muscle preparation. Choline, in the concentration used, itself depressed the force of contraction by 30 to 50%.

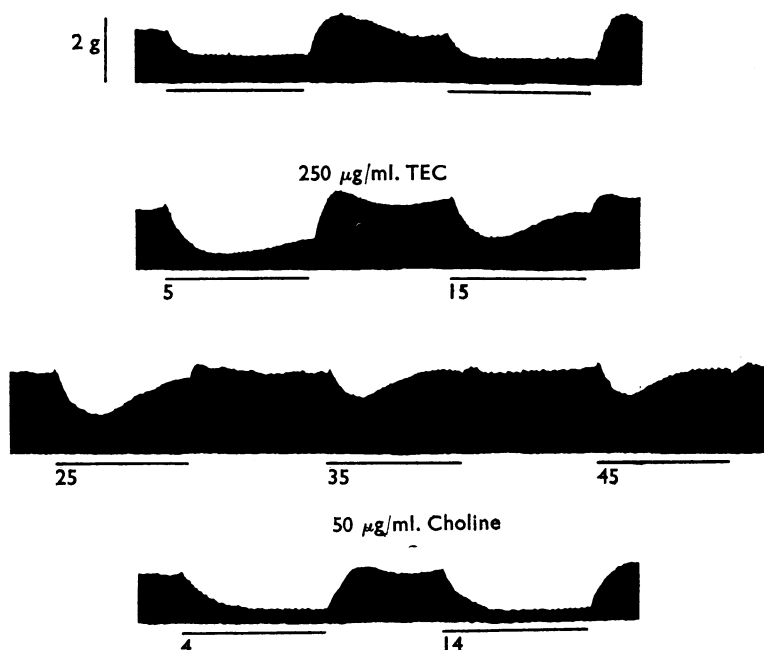


Fig. 6. The actions of triethylcholine on the chick ventricular strip and its responses to field stimulation. The preparation had been previously treated with $20 \mu\text{g/ml}$. guanethidine. The strip was subjected to field stimulation for 5 min periods as indicated by the horizontal lines. The upper record shows 2 control responses. The middle records show responses beginning 5, 15, 25, 35, and 45 min after adding triethylcholine. The bottom record shows responses beginning 4 and 14 min after adding choline without washing out the triethylcholine.

Hexamethonium (up to 1 mg/ml), mecamylamine (up to $20 \mu\text{g/ml}$), or pempidine (up to $40 \mu\text{g/ml}$) had no effect on the inhibitory responses to field stimulation.

Morphine (up to $10 \mu\text{g/ml}$) was also without effect on the depressions obtained with field stimulation.

Field stimulation produced large depressions of the force of contraction in preparations from chicks pretreated with 3 mg/kg reserpine daily for 3 days.

Pigeon, rat and guinea-pig ventricular strips

The results obtained with preparations from chicks were interpreted as evidence for a cholinergic innervation of the right ventricle. An identical method was therefore used on similar preparations from small mammals, since there is disagreement about the extent of the vagal innervation of the ventricle in the mammal (see Discussion). It was also of interest to discover if a cholinergic, inhibitory nerve supply to the ventricle is present in other avian species.

Pigeon

Strips from the right ventricles of 8 pigeon hearts were set up. The application of field stimulation in all untreated strips produced an increase in the force of contraction as in

fowl ventricular strips in the presence of atropine. A 5 msec stimulus pulse duration was more effective in producing an increase in the force of contraction than a 0.5 msec pulse duration.

Increases in the force of contraction occurred with field stimulation when stimulating at 1 to 6 impulses/sec, and these were blocked by guanethidine (1 to 8 $\mu\text{g}/\text{ml.}$) (Fig. 7) or guanoclor (1 $\mu\text{g}/\text{ml.}$), which often had a positive inotropic action. In only one preparation, after blockade of the positive inotropic response to field stimulation, was a depression of the force of contraction produced. This inhibitory response was abolished by 0.1 $\mu\text{g}/\text{ml.}$ atropine and it returned after washing. The strip from the right ventricle of the pigeon heart was very sensitive to acetylcholine (Fig. 8) which produced a swift depression after its addition to the bath. This depression was much more rapid than that observed when using fowl ventricular strips. Depressions in the force of contraction when field stimulation was applied were obtained on 5 strips after first increasing the force of contraction by added noradrenaline (Fig. 7) or calcium chloride. Depressions were greater at 5 msec than at 0.5 msec pulse duration (Fig. 7) and were increased as the stimulus

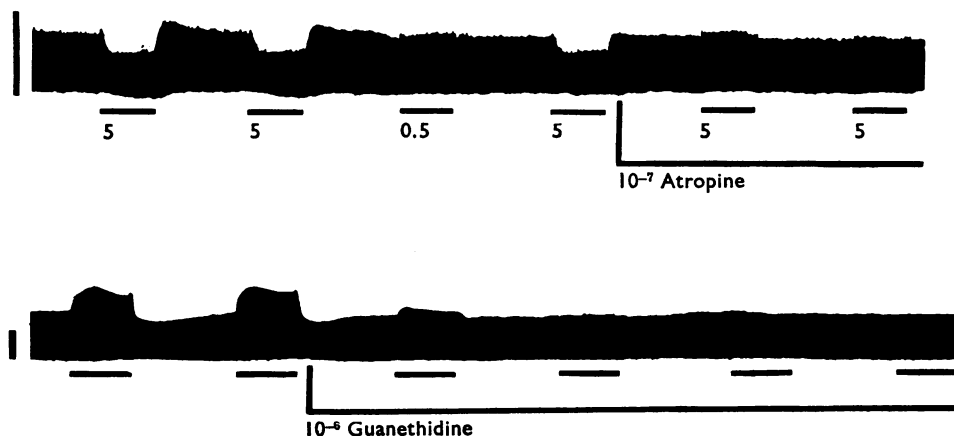


Fig. 7. Upper record: the effect of atropine on the responses of a pigeon ventricular strip to field stimulation. The strip had been previously treated with 20 $\mu\text{g}/\text{ml.}$ guanethidine and 20 ng/ml. noradrenaline is present throughout. Lower record: the action of guanethidine on the responses of an untreated ventricular strip to field stimulation. In both records field stimulation was applied for 90 sec periods as indicated by the horizontal lines. In the upper record the duration of the applied field stimulation is shown in msec.

strength was increased. These depressions were blocked by atropine (0.1 $\mu\text{g}/\text{ml.}$) (Fig. 7) or hyoscine (0.1 $\mu\text{g}/\text{ml.}$). On only 2 preparations was there no evidence of an inhibitory response to field stimulation obtained after augmenting the force of contraction in these ways. Depression in response to field stimulation, like those produced by added acetylcholine, reached a maximum more quickly than in fowl preparations.

Rat and guinea-pig

Similar experiments were made on ventricular strips from 7 adult rats and 11 guinea-pigs of varying ages. In both species, field stimulation caused an increase in the force of contraction which was more marked at 5 msec than at 0.5 or 1 msec pulse duration

and which increased as the stimulus strength was increased. This increase in the force of contraction was blocked by propranolol (0.1 to 1 $\mu\text{g}/\text{ml}$), guanethidine (1 to 10 $\mu\text{g}/\text{ml}$), or guanoxan (1 to 10 $\mu\text{g}/\text{ml}$). The latter two drugs had a positive inotropic action. Strips from both species were very insensitive to acetylcholine, 100 $\mu\text{g}/\text{ml}$ being needed to depress the force of contraction by about 50% (Fig. 8). This is very much less sensitive than the strips taken from the chick or pigeon.

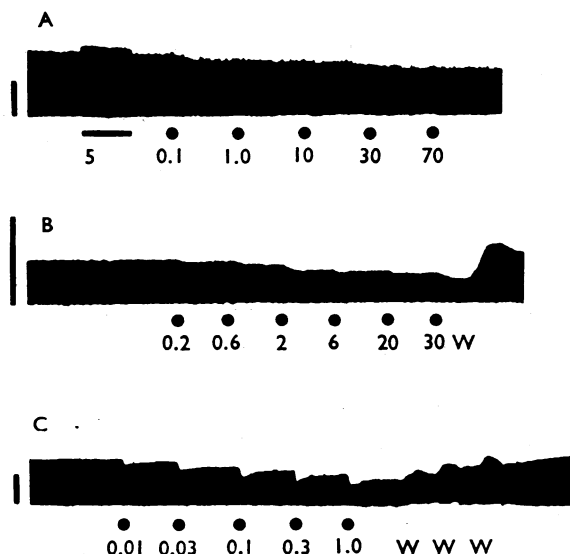


Fig. 8. The action of acetylcholine on ventricular strips from the rat, A, the guinea-pig, B, and the pigeon, C. In A the horizontal line represents a 90 sec period of field stimulation and the first figure the duration (msec) of the applied field pulses. All other figures are the concentrations of acetylcholine (in $\mu\text{g}/\text{ml}$) which were added in a cumulative manner. The tissues were washed (w) as shown. Rat and guinea-pig strips had been previously treated with guanethidine.

In the presence of adrenergic neurone blocking drugs, there was no convincing evidence of an inhibitory response to field stimulation even after augmenting the force of contraction with noradrenaline, a procedure which potentiated or revealed inhibitory responses in the fowl and pigeon ventricles; nor was the sensitivity to acetylcholine increased by this treatment. Occasionally a small reduction in the force of contraction occurred when field stimulation was applied after the addition of adrenergic neurone blocking drugs. Such depressions appeared and disappeared spontaneously and consequently it was not possible to study them.

DISCUSSION

The results provide strong pharmacological evidence that field stimulation of ventricular muscle may excite intramural cardiac nerves. This possibility must be borne in mind when using driven preparations of cardiac muscle, and if necessary, steps should be taken to minimize the effects of any released neurotransmitters.

In most of the present experiments, moderate increases in a just suprathreshold stimulus strength did not change the force of contraction, and it is therefore probable that such stimulus strengths can excite cardiac muscle cells without exciting an appreciable number of nervous elements. Similar conclusions were reached by Koch-Weser (1965) and Blinks (1966). However, a number of authors have reported responses to electrical stimulation of intramural nerves when the stimulus applied was subthreshold for the cardiac muscle cells (Nelemans, 1951; Lewartowski & Bielecki, 1963; Vincenzi & West, 1963).

Evidence that the positive inotropic effect of field stimulation in all species studied was due to stimulation of intramural adrenergic nerves is derived from the findings that it was blocked by the usual effective concentrations of β -receptor blocking drugs, and by adrenergic neurone blocking drugs in concentrations similar to those found effective in blocking the responses of cardiac muscle to extrinsic adrenergic nerve stimulation (Huković, 1960; Day & Rand, 1961).

The fowl and the pigeon ventricular strips were sensitive to acetylcholine and the depressions obtained with field stimulation were blocked by atropine and hyoscine and impaired by TEC and TEA, the effects of TEC being well reversed by choline. These observations constitute strong evidence that the right ventricle of these avians possesses a cholinergic, probably vagal, innervation. Field stimulation of the fowl ventricular strip often produced marked depressions even when adrenergic nerves were being stimulated simultaneously, indicating that the cholinergic innervation is well developed and can probably exert a marked direct action on ventricular function *in vivo*.

There was little evidence for a similar cholinergic innervation in the cases of the rat and the guinea-pig. Ventricular tissue from these two species was much less sensitive to acetylcholine, as has also been found with ventricular tissue of other mammals (Bozler, 1942; Brooks, Hoffman, Suckling & Orias 1955; Benforado, 1958), although the resting and action potentials of ventricular muscle from the frog and toad are profoundly altered by acetylcholine (Brooks *et al.*, 1955; Azuma, Hayashi & Matsuda, 1962).

Vincenzi & West (1963) found no evidence of a vagal innervation in 5 guinea-pig ventricular strips subjected to field stimulation, and results described in this paper confirm their finding. In the present experiments, the apparent absence of any functional cholinergic nerve effects in the ventricles of rat and guinea-pig is made more convincing, since the experiments were performed under conditions identical with those in which cholinergic nerve effects were readily demonstrable in chick and pigeon ventricles.

No pharmacological evidence for the presence of ganglion cells in the right ventricle of the chick was obtained. Thus, the ganglion blocking drugs hexamethonium, mecamlamine and pempidine were without effect on both types of response to field stimulation. Some ganglion stimulant drugs—viz., TMA, McN-A-343, and AHR-602—depressed the force of contraction of driven chick ventricular muscle, but this response, although blocked by atropine, was unaffected by ganglion blocking drugs indicating that it was the result of the known muscarinic action of these ganglion stimulants (Roszkowski, 1961; Franko, Ward & Alphin, 1963; Barlow, 1964). The ganglion stimulants, nicotine and DMPP, which have *not* been shown to possess muscarinic activity, did not depress the contractions of driven chick ventricle.

Hsieh (1951) who worked on adult fowls described discrete nerves extending on to the base of the ventricles. His drawings show that the anterior cardiac branch of the left vagus nerve is clearly distinguishable until it passes beyond the atrioventricular groove where it divides and becomes part of the anterior cardiac plexus. This plexus contains ganglia, and the post-ganglionic cholinergic fibres found in the ventricle must originate from these ganglia.

It is probable that, in both fowl and mammal, ganglia are mainly confined to the nerve plexuses which occupy a superficial position covering the atria in the mammal, and the atria and base of the ventricles in the fowl. In the fowl, post-ganglionic fibres pass from these ganglia to all regions of the ventricle. In the mammal these post-ganglionic fibres probably do not pass more apically than the base of the ventricles.

After pretreatment of chicks with reserpine, adrenergic nerve activity still exerted a positive inotropic action on ventricular muscle, despite the very marked increase in the basal force of contraction which reserpine produced. Thus the cardiac adrenergic nerves of the chick resemble those innervating the guinea-pig atrium, as their activity was not abolished by pretreatment with reserpine (Trendelenburg, 1965; Blinks, 1966), rather than those to the rabbit atrium (Huković, 1959; Trendelenburg, 1965) and to the kitten atrium (Blinks, 1966) which fail to exert any effect after reserpine pretreatment. The increased basal force of contraction following reserpine pretreatment was not due to a continuous release of catecholamines, as doses of adrenergic neurone blocking drugs and adreno-receptor blocking drugs, which completely abolished the effects of adrenergic nerve stimulation, did not affect the basal force of contraction. It is therefore concluded that the increase in the force of contraction of chick ventricular strips following reserpine pretreatment was due to a direct action of the drug on the cardiac muscle cells.

In the mammal the effects of noradrenaline and of adrenergic nerve stimulation are potentiated by cocaine. However, in the fowl ventricular strip cocaine did not potentiate the effects of stimulating adrenergic nerves or of noradrenaline. In earlier experiments on isolated perfused chick hearts, 1 to 2 $\mu\text{g/ml}$. cocaine reduced the positive chronotropic effect of adrenaline but increased its duration of action (Bolton, 1967). Adrenergic neurone blocking drugs and phenoxybenzamine potentiated the responses of chick ventricular muscle to noradrenaline.

The action of TEC on the cholinergic nerves of the ventricular strip showed many similarities to its action on the somatic nerves to skeletal muscle. TEC and TEA initially facilitate the release of acetylcholine (Bowman & Rand, 1961; Roberts, 1962; Bowman & Hemsworth, 1965) and this probably explains why these drugs potentiated the initial depression obtained upon cholinergic nerve stimulation in these experiments. The observed slow onset of transmission block and partial recovery of nerve function when rested are characteristic of a prejuncional type of blockade (Bowman & Rand, 1961; Bowman *et al.*, 1962). The prejuncional action is also confirmed by the reversal of block with choline.

Many compounds related to TEC possess atropine-like activity (Holton & Ing, 1949) and it is possible that TEC does also. However, the block by atropine in the ventricular strip preparation was virtually complete 5 min after its addition. In contrast, the block by TEC was much slower in onset and 5 min after adding TEC, cholinergic nerve activity was often potentiated. Choline and acetylcholine failed to depress the force of contraction

after blockade with small doses of atropine although after a similar degree of blockade by TEC, both drugs invariably depressed the force of contraction. It is concluded that TEC blockade of the cholinergic nerves to the ventricle was not due to its atropine-like activity but was caused by an inhibition of acetylcholine synthesis.

Local anaesthetics did not generally selectively block nervous transmission without a direct effect on the cardiac muscle, although procaine did block cholinergic nerve effects in concentrations which had an insignificant influence on the basal force of contraction. In these experiments, procaine antagonized cholinergic nerve effects more effectively than those due to added acetylcholine, possibly suggesting that procaine was exerting some action on the nerve terminals. However, hyoscine had a very similar action to that of procaine and it is probable that residual adrenergic nerve activity is the explanation. If this is true the blockade of the responses to cholinergic nerve stimulation would be wholly due to the atropine-like activity of procaine described by others (Dawes, 1946; de Elío, 1948).

Increasing the duration of the applied stimuli, without any change in the rate of stimulation, sometimes produced a contracture. If the depolarization plateau of the cardiac action potential is extended by applying a strong electrical current, the ventricular muscle cells, but not atrial muscle cells, remain in a contracted state. When repolarization is allowed to occur the tension falls (Kavalier, 1959, 1960). The contractures observed here could be due to a prolongation of the depolarization by the long stimulus pulse durations; hence the next contraction would begin before complete relaxation had occurred. However, contractures were slow in onset and declined over several minutes, unlike the clonus or tetanus observed in ventricular tissue from the frog or mammal (Whitehorn, 1954; Rosin & Farah 1955) which begin and end much more abruptly. A more likely explanation is that the increased duration of the applied stimuli, combined with the high current strength, activate the contractile components of the cardiac muscle cells in some unusual way. It is possible that high strength electrical fields release bound ions, such as calcium, which then activate the contractile proteins. A similar explanation has been invoked by Sperelakis (1962) to explain the contractures observed in depolarized smooth muscle subjected to alternating, high strength, electrical fields.

SUMMARY

1. Pharmacological evidence was obtained which showed that field stimulation, applied to electrically driven strips of right cardiac ventricular tissue from the fowl, the pigeon, the guinea-pig and the rat, excites intramural cardiac nerves.

2. The ventricle of the fowl and pigeon were shown to contain both adrenergic and cholinergic nerves, whereas only an adrenergic innervation was present in preparations from the guinea-pig and rat. No evidence for the presence of autonomic ganglia in the fowl ventricular muscle preparation was obtained.

3. The effects of adrenergic nerve stimulation were abolished by β -receptor blocking drugs and by adrenergic neurone blocking drugs. Reserpine pretreatment of the fowl did not abolish the effects of adrenergic nerve stimulation and it more than doubled the force of contraction of the driven ventricular strip, probably through a direct action on

the cardiac muscle. Cocaine did not potentiate the effects of adrenergic nerve stimulation or of noradrenaline.

4. The effects of cholinergic nerve stimulation in the fowl ventricle were abolished by atropine and hyoscine, and by triethylcholine. The effect of triethylcholine was reversed by choline.

5. The local anaesthetics lignocaine, cinchocaine and cocaine reduced responses to nerve stimulation only in concentrations which also reduced the activity of the cardiac muscle cells. Procaine blocked the responses to cholinergic nerve stimulation and to acetylcholine as a result of an atropine-like action.

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