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A METHOD OF STIMULATING THE COMPLETE SYMPATHETIC OUTFLOW FROM THE SPINAL CORD TO BLOOD VESSELS IN THE PITHED RAT

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The effects of sympathetic nerve stimulation on blood vessels have been studied in a variety of ways. The vascular beds of various organs or regions have been perfused either *in vivo* or *in vitro* with blood or saline and the pressor response to stimulation of the operatively exposed sympathetic nerves measured. In other experiments the response of isolated arteries to stimulation of their sympathetic nerves has been studied. None of these methods gives information on the integrated response of the blood vessels constituting the peripheral resistance to stimulation of the entire sympathetic outflow. Furthermore, in experiments stimulating exposed sympathetic nerves, the operative interference in making the preparation may itself affect the vascular responses.

The technique here described involves no further operative interference beyond that involved in pithing the animal and recording blood pressure, and the pressor responses obtained probably involve the entire vasculature and its sympathetic nerve supply. In principle, the steel pithing rod is used as one electrode to stimulate spinal nerve roots. Large rises in arterial blood pressure can be produced and the method is particularly suited to studying the action of drugs.

METHODS

Rats (150–350 g) were anaesthetized with ether following atropine (1 mg/kg intraperitoneally). The trachea was cannulated, and animals pithed through one orbit with a steel rod and immediately artificially respired. Blood pressure was recorded from a carotid artery using a Condon mercury manometer and one femoral vein was cannulated for the administration of drugs. Those parts of the pithing rod which lay in the sacral and cervical regions of the spinal cord were coated with high-resistance varnish to restrict stimulation to the thoraco-lumbar region. The appropriate parts of the rod to be coated were determined by exposing the pithing rod as it lay in the vertebral canal of a rat of similar size. In all animals excitation included the upper lumbar region as shown by twitching of the lower limbs. Any residual effects of stimulating sacral or vagal parasympathetic fibres by spread of the stimulating current were eliminated by a second injection of atropine (1 mg/kg intravenously). In preliminary experiments, premedication with atropine was omitted and the effect of this drug on the vasopressor response to nerve stimulation studied. Atropine did not reduce the pressor response (Fig. 1A). Electrical stimulation affects the motor fibres in the ventral roots, causing twitching of skeletal muscle. In addition to interfering with respiration, these twitches on

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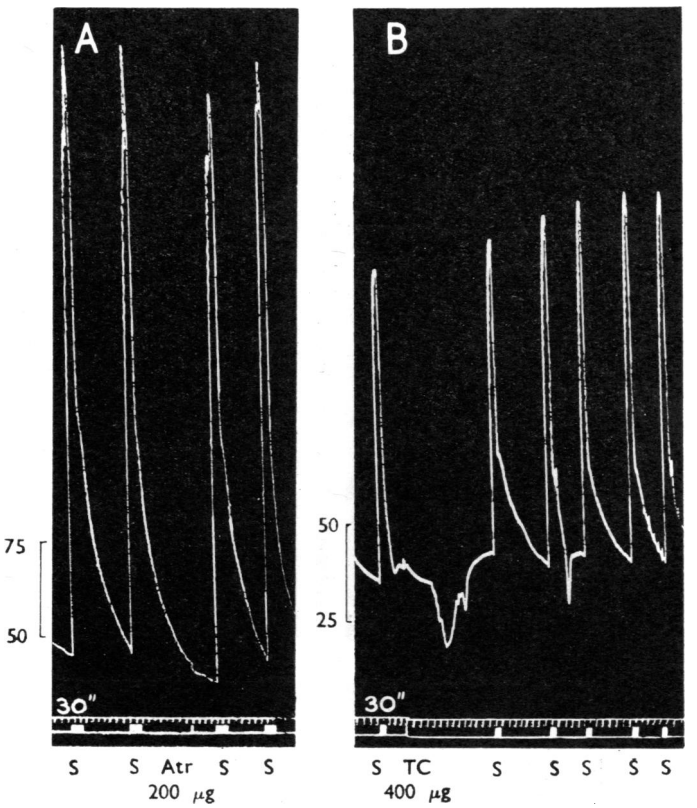

Fig. 1A. The effect of atropine (Atr) on the response of the blood pressure of a pithed rat (250 g) to supramaximal nerve stimulation of the sympathetic outflow (S) for 30 sec at 10/sec. Atropine does not block the response, other than a slight temporary reduction corresponding to a fall in the base line. In Fig. 1B in another rat (250 g) in the presence of atropine (300 μg intraperitoneally) tubocurarine increased the response to nerve stimulation at a frequency of 5/sec, probably by improving respiration after abolishing the effect of nerve stimulation on the respiratory muscles. In this and subsequent figures, the ordinate scale refers to blood pressure in mm Hg. In all subsequent figures atropine (2 mg/kg) and tubocurarine (400 μg) were present throughout.

occasion dislodged the arterial or venous cannula. The twitches could be abolished by tubocurarine (1–3 mg/kg, intravenously). The possibility that ganglion blockade by tubocurarine might reduce the pressor response was investigated. No evidence of ganglion block was found (Fig. 1B).

The spinal cord was stimulated electrically by 1 msec supramaximal pulses at the frequencies indicated in the text. The pithing rod served as the stimulating electrode and a length of silver wire under the skin in the femoral region as an indifferent electrode. Several other sites for the indifferent electrode were tested; within the oesophagus or rectum and under the skin of the neck. In all sites, continuous stimulation over long periods affected the heart, as shown by a fall in blood pressure and the appearance of extra systoles. These effects were least evident when the indifferent electrode was placed in the leg.

The following drugs were given intravenously and the doses refer to the salts. (−)-Adrenaline bitartrate (B.D.H.), atropine sulphate (B.D.H.), (—)-noradrenaline bitartrate (Koch-Light), guanethidine monosulphate (Ciba), hexamethonium bromide (May & Baker), (±)-pronethalol hydrochloride (I.C.I.) and (+)-tubocurarine chloride (Burroughs Wellcome).

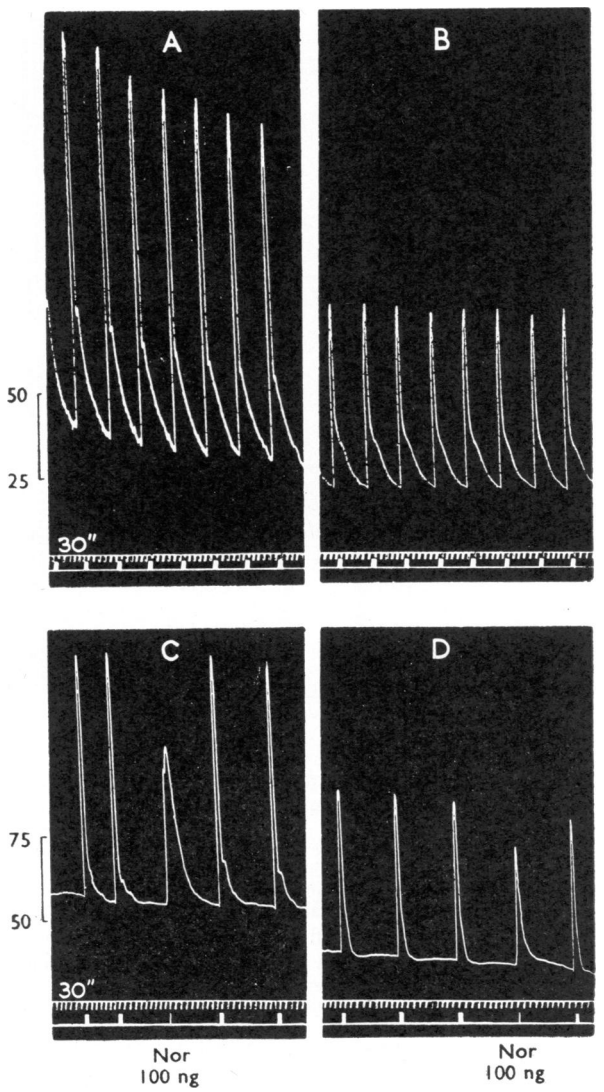

Fig. 2. The response of the blood pressure of the pithed rat (250 g) to supramaximal sympathetic nerve stimulation (at the signal marker) and to 100 ng of noradrenaline (Nor). A and B show the effect of 20 sec periods of stimulation at 5/sec at 3 min intervals. In the 45 min between A and B these periods of nerve stimulation continued. The response declined as shown in A to reach the stable value in B. In C and D in another rat (250 g), the periods of nerve stimulation (2/sec) were separated by intervals of 5 min. Between C and D is an interval of 95 min in which periods of stimulation continued. The rate and extent of the decline with time is less; the response to noradrenaline is also reduced.

RESULTS

Pressor response to short periods of stimulation

Short periods of stimulation at a frequency of between 2 and 10/sec were repeated at intervals of 3 to 5 min. The duration of stimulation, usually 20–30 sec, was such as to allow the maximum pressor response to be reached.

There was a rapid rise in pressure averaging in 20 experiments 105.6 ± 4.5 mm Hg at 10/sec and in individual experiments this could be as high as 200 mm Hg. The results of one experiment are shown in Fig. 2. Following the end of stimulation, the blood pressure fell in two phases. First, a rapid fall comparable with the rate of rise of pressure followed by a slower decline to the previous level (Fig. 2A). The slow phase in the decline was variable as can be seen by comparing D and A in Fig. 2, and is probably due to circulating catecholamines from the adrenal glands, since removal of these abolished this phase. The height of the individual pressor responses declined with time over the first 25–30 min but thereafter remained constant for at least 1 hr. The rate and extent of the decline was influenced both by the frequency of stimulation and the interval between stimulation periods. The higher the frequency and the shorter the rest periods, the more rapid and greater the decline in the response. The response to noradrenaline was usually also reduced (Fig. 2).

The effect of the strength of the stimulating current was studied. A threshold response was obtained usually between 5–15V and thereafter increases in the strength of current produced graded increases in the response until a maximum was reached, usually between 70 and 80V (Fig. 3).

The effect of varying the frequency of stimulation at supramaximal voltage was also studied and the results of one experiment are shown in Fig. 4. Single pulses (1 every 90 sec) produced discrete pressor responses which returned to the base line between shocks. Summation of the response with a maintained rise in pressure above the base line was first apparent at a stimulation frequency of above 1 in 16 sec. The responses to individual pulses were still visible on the raised pressure level and this level was maintained undiminished to the end of the stimulation (Fig. 4). Further increase in the frequency of stimulation caused a progressive rise in the level of the pressure reached, and at a frequency of 1 in 4/sec and above a new feature appeared; the initial rise in pressure was not maintained but declined to a lower value which was then fairly constant until the end of stimulation. With further increase in the frequency of stimulation to approximately 20/sec, the initial pressor responses continued to increase but with little comparable increase in the maintained response. In some instances there was almost no increase in this part of the response as the frequency was raised.

Submaximal responses of constant height could be produced at a given frequency by varying the number of stimuli. At 10/sec maximal responses were obtained with between 200 and 400 pulses; trains of 50 pulses at this frequency gave constant responses for periods up to 2 hr with intervals as short as 3 min (Fig. 5). With these short trains of pulses (5–50 at 10/sec), the first few responses showed a progressive increase in amplitude and the response then remained constant for long periods. There was no evidence of the initial decline observed when 30 sec periods of stimulation were used.

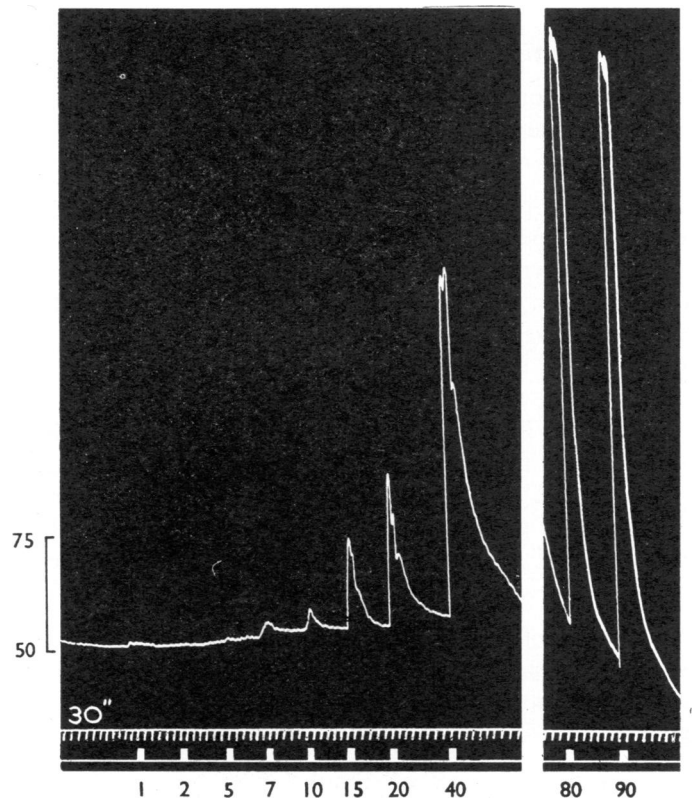

Fig. 3. The effect of increasing the strength of the stimulating current on the response of the blood pressure of the pithed rat (150 g) to sympathetic nerve stimulation. The voltage of the stimulating pulses is shown below the signal marker. Time between panels was 3 min.

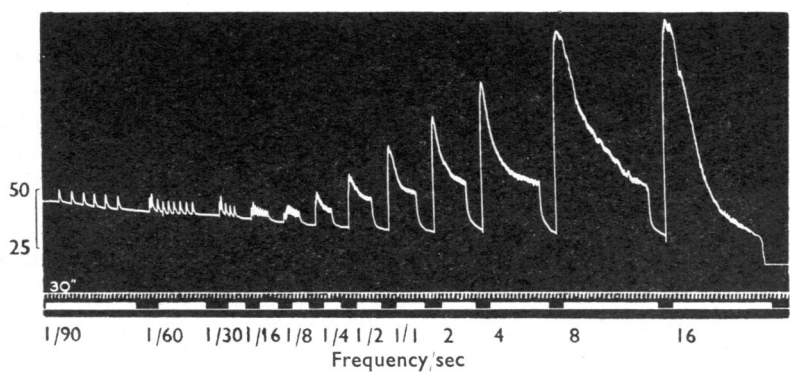

Fig. 4. The effect on the response of the blood pressure of the pithed rat (150 g) of varying the frequency of supramaximal sympathetic nerve stimulation. The duration of stimulation is indicated by the signal marker and the frequency is shown below.

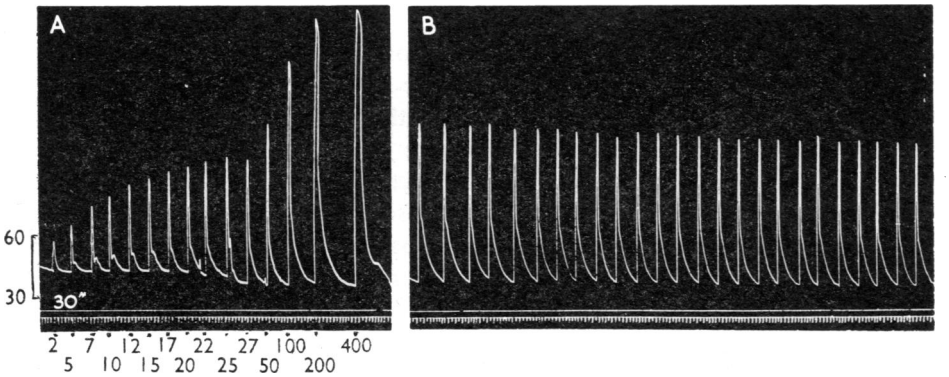

Fig. 5A. The effect on the blood pressure response of a pithed rat (250 g) of varying the number of stimuli at a fixed frequency of 10/sec. The number of stimuli in the train is shown beneath each response. Fig. 5B. The submaximal response to trains of 50 stimuli repeated at 3 min intervals remains constant for 2 hr.

Response to prolonged stimulation

In these experiments, stimulation was continued after the peak response was reached. The results of one experiment at a stimulation frequency of 10/sec are shown in Fig. 6. The initial rise in pressure was not maintained and eventually fell to near the pre-stimulation value. This does not imply, however, that stimulation had ceased to be effective, since, when stimulation was stopped, the pressure fell further and the animals frequently died (Fig. 6).

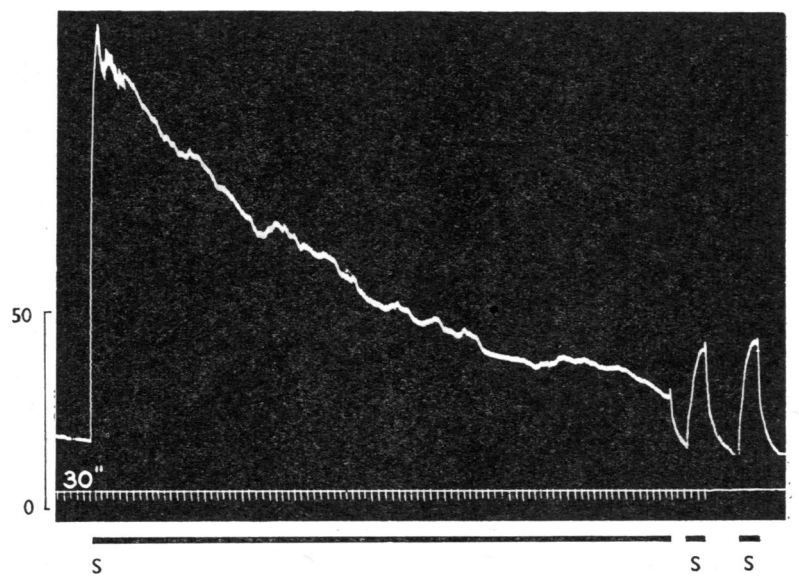

Fig. 6. The blood pressure response of the pithed rat (300 g) to prolonged stimulation of the sympathetic nerves (S) at a frequency of 10/sec as indicated by the black bar below the trace. There is a progressive fall in pressure. Short test periods of nerve stimulation within 1 min of the end of this stimulation period showed some reversal of this decline.

Effects of Drugs

Hexamethonium and guanethidine. From the position of the stimulating electrode, the fibres likely to be affected were the preganglionic sympathetic fibres leaving the spinal ventral roots. This was confirmed by showing that hexamethonium (6 mg/kg intravenously) almost abolished the pressor response (Fig. 7A). Guanethidine (8 mg/kg intravenously), as would be expected, also almost abolished the response presumably by acting on post-ganglionic nerve fibres. The residual effect of nerve stimulation after this drug was very resistant to block by further doses of guanethidine but was abolished by hexamethonium. The response to noradrenaline, after guanethidine, was enhanced (Fig. 7B).

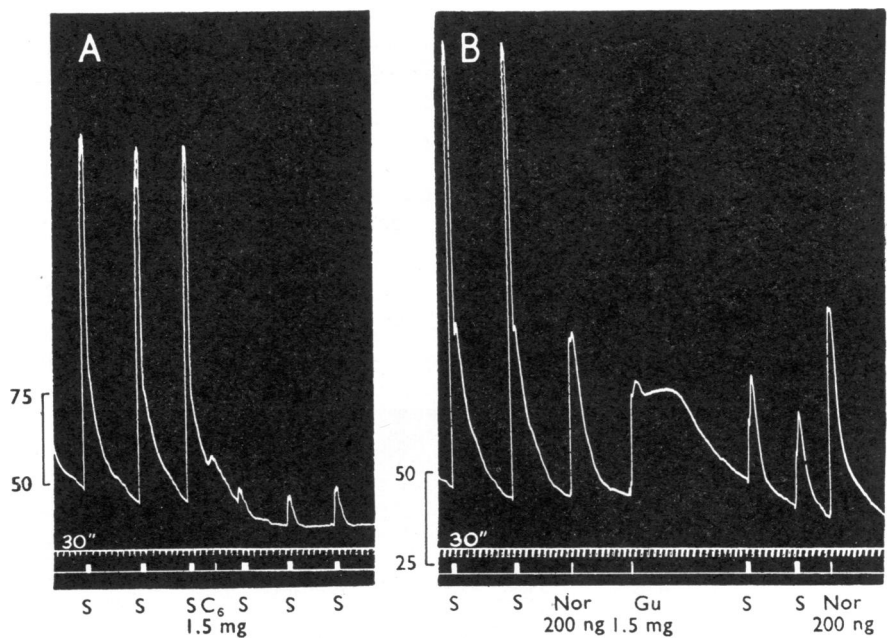

Fig. 7. The effect of hexamethonium (C_6), guanethidine (Gu) and noradrenaline (Nor) injected intravenously on the response of the blood pressure of pithed rats to stimulation of the sympathetic outflow at a frequency of 5/sec. The weights of the animals in A and B were respectively 250 g and 175 g.

Pronethalol. The pressor response to continuous stimulation declines sharply especially at high frequencies (Figs. 4 and 6). The decline might be due to stimulation of β-receptors in smooth muscle, perhaps by adrenaline from the adrenals competing with the constrictor effect produced by stimulation of α-receptors. The action of a β-blocking agent, pronethalol, was examined, therefore, to see if it would prevent the decline. The results of one experiment are shown in Fig. 8. In doses up to 10 mg/kg, pronethalol had no effect either on the response to short (30 sec) periods of stimulation or in preventing the decline in the response to continuous stimulation.

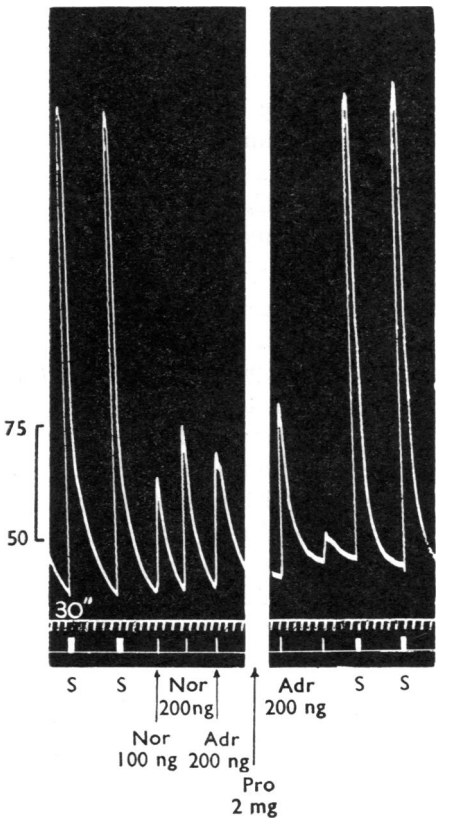

Fig. 8. The effect of pronethalol (Pro) on the response of the blood pressure of a pithed rat (200 g) to stimulation of the sympathetic outflow (S) for 20 sec at a frequency of 20/sec. At Nor, and Adr respectively, noradrenaline and adrenaline were injected intravenously in the doses indicated. Time between panels was 5 min. The unmarked pressor rise in the second panel is a saline artefact (0.4 ml.).

DISCUSSION

The pressor response to stimulation of the thoraco-lumbar spinal nerve roots is clearly mediated through an adrenergic mechanism and the action of hexamethonium suggests that it is due to stimulation of preganglionic fibres. The shape of the response, and the effect of adrenalectomy, suggests that both adrenergic nerves and the adrenal glands are involved. Since guanethidine, which acts on terminal adrenergic nerve fibres, almost abolished the response, while the β-blocking agent pronethalol had no effect, the part played by the adrenals or adrenaline appears to be small. The observations that hexamethonium abolished the residual response in the presence of guanethidine suggests that it is this component which is due to the adrenals.

The extent of the sympathetic outflow stimulated was difficult to determine without measuring the effect on many isolated vascular beds. The most likely error would be to miss one or other end of the thoraco-lumbar outflow due to the position of the varnish

on the rod, or because the rod was not fully inserted. In all experiments, the lower limbs were observed to twitch during stimulation, indicating that current was reaching the mid-lumbar region and should, therefore, involve all of the lumbar sympathetic outflow In experiments described in the following article, one lower limb was perfused with a constant volume output pump. Nerve stimulation caused large rises in pressure in this preparation, confirming that the most caudal sympathetic outflow was stimulated. Finally, the magnitude of the response, which reached 200 mm Hg on occasions from an initial level of 30 mm Hg, suggested that most, if not all of the sympathetic outflow, was affected.

During prolonged stimulation at frequencies above 1 in 4/sec, the initial pressor response declined to a steady maintained level; this fall was greater the greater the frequency of stimulation, so that the maintained level was almost independent of the frequency employed. One explanation is that, at high frequencies, the available stores of noradrenaline are exhausted during the initial response. During this period, the response is related to the frequency of stimulation. When this store is used up, the rate of release is determined by the rate at which noradrenaline can be made available, either by transfer from another store, or by synthesis, and so is no longer related to frequency. A somewhat similar mechanism operates at cholinergic junctions in ganglia (Perry, 1953; Birks & MacIntosh, 1961). However, evidence is presented in the following article that the magnitude of this maintained phase of the response is, at least, partly determined by the ability of the heart to maintain the pressure level. It is doubtful, therefore, whether it can be used as a measure either of the amount of noradrenaline released or of the response of the vascular smooth muscle. The initial rapid rise in pressure is related to the frequency of stimulation (Fig. 4). It is this component which is measured when short test periods of stimulation are used. The most consistent responses and the least evidence of damage to the preparation were obtained with short (10–50) trains of pulses at 10/sec, and this would be the most useful technique in analysing the site and mode of action of drugs. The value of this preparation would have been greatly enhanced if it had also been possible to stimulate post-ganglionic fibres. Two methods of doing this were tried. First, the strength of the stimulating current was increased in the presence of hexamethonium in the hope that current spread would reach the ganglion. Secondly, a silver wire was slipped from the neck down the tissue plane between oesophagus and vertebral column, passing through the diaphragm with the oesophagus. This can be done without harming the preparation as judged by the blood pressure. Stimulation was applied between this wire and either the indifferent electrode or the pithing rod. Neither method was successful in stimulating post-ganglionic fibres.

SUMMARY

1. A method of stimulating the entire spinal vasomotor outflow in the pithed rat is described. The steel rod in the vertebral canal was used as one electrode with an indifferent electrode under the skin.

2. With short bursts of stimulation graded responses reproducible over long periods could be obtained either by varying the frequency of stimulation or the number of stimuli in a train.

3. The response to prolonged stimulation depended on the frequency. At frequencies below 2/min discrete responses to each stimulus were observed ; above this, the responses fused to give a maintained rise of 10–20 mm Hg. At still higher frequencies above 1/sec the initial rise increased with increasing frequency but was followed by a decline to a lower level which was then maintained constant till the end of stimulation. The maximum response was reached at about 16/sec.

4. Hexamethonium (6 mg/kg) almost abolished the rise in pressure. Pronethalol (10 mg/kg) did not alter the response. Guanethidine (8 mg/kg) reduced the pressor response. In the presence of guanethidine, the residual pressor response was abolished by hexamethonium and may, therefore, be due to the release of catecholamines from the adrenal gland.

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