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Depressor responses to spinal stimulation in the pithed rat

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Summary

1. Electrical stimulation of the spinal nerves in the pithed rat preparation produces a pressor response due to sympathetic vasoconstriction.

2. When the vasoconstrictor effect of sympathetic stimulation is abolished by guanethidine or hexamethonium and the blood pressure is raised by noradrenaline infusion, spinal stimulation produces depressor responses or complex responses containing depressor components.

3. Contractions of skeletal muscle caused by stimulation of motor nerves result in complex changes in blood pressure consisting of a pressor component due to clamping of muscle blood vessels and a secondary depressor phase due to functional hyperaemia.

4. The depressor response is partly due to stimulation of cholinergic postganglionic fibres. The acetylcholine released, which causes vasodilatation, may be the overflow from neuromuscular junctions or ganglionic synapses.

5. Stimulation of the nerves to the adrenal medulla causes release of adrenaline which has a vasodilator effect during noradrenaline infusion.

Introduction

Gillespie & Muir (1967a) described a preparation of the pithed rat in which the cardiovascular effects of stimulation of the entire sympathetic outflow from the spinal cord can be observed. Sympathetic stimulation caused only a rise in blood pressure which was shown to be due to vasoconstriction mediated by vasomotor sympathetic nerves or by circulating catecholamines released from the adrenal medullae (Gillespie, Maclaren & Pollock, 1970).

With continued stimulation, the pressor response declined and on cessation of prolonged stimulation blood pressure fell below the previous control level: these effects were attributed to the failure of the heart to maintain an adequate output (Gillespie & Muir, 1967a, b). Another possible explanation for the failure to maintain a sustained rise of pressure with continued sympathetic stimulation is that counteracting depressor activity is developed; although Gillespie & Muir (1967b) have shown that there was sustained vasoconstriction in the hindquarters, there may be vasodilatation in other vascular beds.

We set out to explore the possibility that spinal stimulation may produce depressor effects. However, in the pithed rat the blood pressure is low and the preparation is extremely sensitive to pressor agents (Shipley & Tilden, 1947). Therefore, observations were made in rats in which the blood pressure was raised by infusions of pressor agents and in which the sympathetically mediated vasoconstriction was blocked with guanethidine.

Methods

Sprague-Dawley albino rats in the narrow weight range of 200 to 220 g were prepared as described by Gillespie & Muir (1967a), but no atropine or tubocurarine was given. The metal pithing rod, which is one stimulating electrode, was a tight fit in the vertebral canal and had a mark to indicate the correct depth for insertion. Dissection of several preparations showed that the tip was located in segments L5 to L6 of the vertebral column. The portion cephalal to T1 was coated with varnish. The second electrode was inserted into the muscle of one hind leg. The pithed rats were ventilated with a Palmer respiration pump at a rate of 90–100/min and stroke volume of 2·5 to 3 ml. Blood pressure (1 mmHg≡1·33 mbar) was measured from the left carotid artery with a Statham pressure transducer and recorded on Beckman-Offner Dynographs with curvilinear or rectilinear pens. A femoral vein was cannulated for injections and left jugular vein was cannulated for infusions of drugs. Some rats were adrenalectomized by plucking out the adrenal glands with forceps through lateral incisions.

Spinal stimulation was applied from a Palmer square wave stimulator at maximal voltage (80–100 V) at a frequency of 5 Hz for periods of 10 s at intervals of not less than 5 minutes. The effects of a range of pulse widths were studied but in most experiments two pulse widths of stimulation were chosen: short pulses of 0·1 ms were used to stimulate large diameter fibres selectively; longer pulses of 1 ms were used to ensure stimulation of small diameter fibres.

In order to raise the blood pressure, to provide the potential for depressor effects to be exhibited, an infusion of noradrenaline was given. In preliminary experiments it was established that (25 μg/kg)/min infused in 0·025 ml of solution per min gave satisfactory results.

The following drugs were used: acetylcholine bromide (Roche), atropine sulphate (Macfarlan Smith Ltd.), dipyridamole (Boehringer Ingelheim), gallamine triethiodide (May & Baker), guanethidine sulphate (Ciba), hexamethonium bromide (May & Baker), neostigmine bromide (Roche), (−)-noradrenaline bitartrate (Winthrop), (±)-propranolol hydrochloride (I.C.I.), (+)-tubocurarine chloride (Burroughs Wellcome). The doses given in the text refer to these salts.

Results

Effect of a noradrenaline infusion on responses to spinal stimulation

Pressor responses to spinal stimulation (1 ms pulses) consisted of a rapid and transient rise followed by a slower decline to baseline after cessation of stimulation, as reported by Gillespie & Muir (1967a). During an infusion of noradrenaline which raised the blood pressure from about 25 to 180 mmHg, the pattern of the response to spinal stimulation changed (Fig. 1). There was an initial transient fall followed by a rise in pressure during the period of stimulation; after cessation of the stimulation there was a rapid drop in pressure which decayed into a slowly developing depressor phase with recovery to the pre-stimulation level in about 5 minutes. Hexamethonium abolished the pressor component of the complex response, leaving a purely depressor response with a rapid initial fall and a subsequent slow return to the pre-stimulation level.

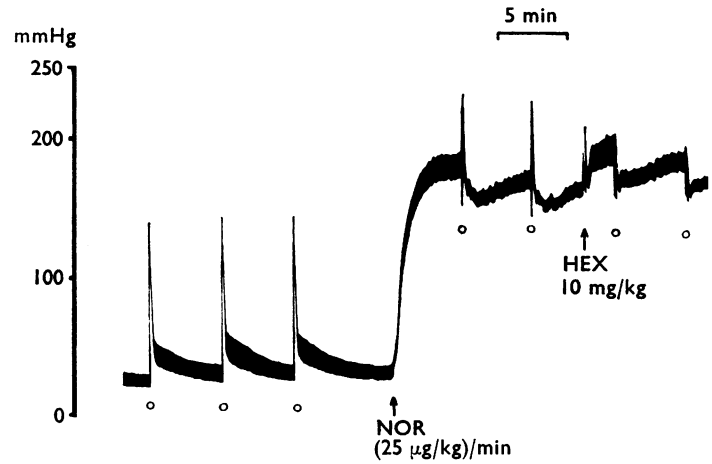

FIG. 1. Pressor responses to spinal stimulation before and during noradrenaline infusion (NOR). Stimulation with 1 ms pulses at 5 Hz for 10 s is indicated at O. HEX=hexamethonium. The record was made with a rectilinear pen.

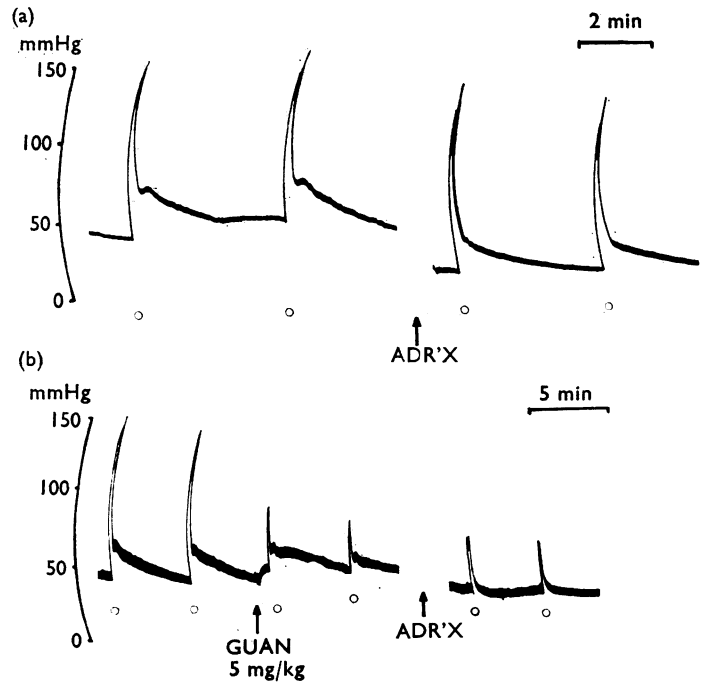

FIG. 2. Effect of adrenalectomy (ADR'X) on the residual pressor response: (a) in an untreated rat; (b) after guanethidine (GUAN). Spinal stimulation was given at O with 1 ms pulses at 5 Hz for 10 s. A curvilinear recorder was used.

Effect of guanethidine on responses to spinal stimulation

Guanethidine in doses of 1 to 5 mg/kg markedly reduced the initial transient phase of the pressor response to spinal stimulation (1 ms pulses), but the secondary slowly declining phase was only slightly affected. The residual pressor response after guanethidine was attributed by Gillespie & Muir (1967a) to release of

catecholamines from the adrenals. After guanethidine, adrenalectomy abolished the secondary pressor phase, but the residual primary pressor response was not affected (Fig. 2b). In the absence of guanethidine, the secondary pressor phase was reduced but not abolished (Fig. 2a).

Pulse width of spinal stimuli

Gillespie & Muir (1967a) investigated the effects of varying the strength of the pulses, the frequency and the duration of trains of stimulation, but did not report on the effects of varying the pulse width. The pressor response was nearly maximal with pulse widths of 0·5 ms (Fig. 3); with pulse widths above 2 ms, the responses were less than maximal. The pressor response to stimulation with pulse widths of less than 0·5 ms was not followed by a secondary pressor phase. In three experiments after guanethidine, the residual pressor response changed only slightly with increase of pulse width from 0·02 ms to 1 ms, but with pulses of 2 ms or more the pressor response increased. Pulse widths of 0·1 ms and 1 ms were selected for subsequent experiments. Hexamethonium (10 mg/kg) reduced the pressor response to spinal stimulation with either pulse width to about the same extent as did guanethidine and abolished the secondary pressor phase of responses to stimulation with the longer pulse width.

Spinal stimulation during noradrenaline infusion in guanethidine treated rats

Noradrenaline infusion ((25 μg/kg)/min) in guanethidine-treated rats produced a rise of blood pressure to 100 to 200 mmHg. The rise was well maintained for periods of up to 3 h, in contrast to the decline in pressure produced by infusions in rats not treated with guanethidine (Gillespie & Muir, 1967b).

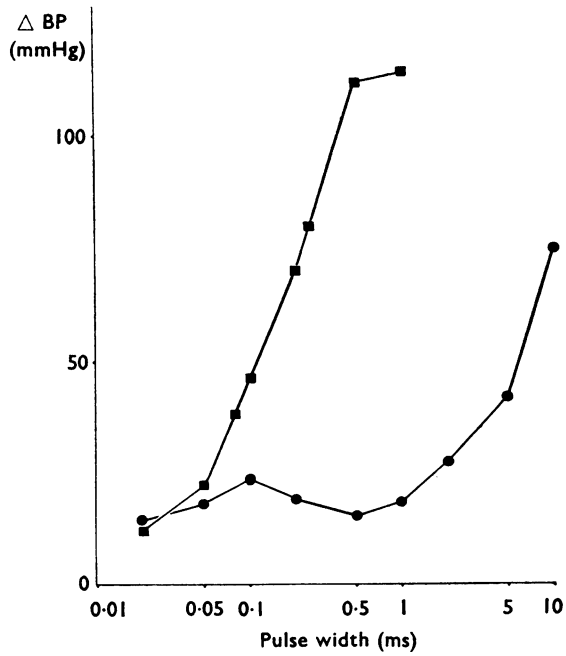

FIG. 3. Relation between pulse width (in ms on abscissa) and pressor response to spinal stimulation in the absence (■) and presence (●) of guanethidine (1 mg/kg). Results of one experiment are shown. Stimulation was given at 5 Hz for 10 s periods at intervals of not less than 5 min.

Spinal stimulation with either short (0·1 ms) or long (1 ms) pulses during noradrenaline infusions in guanethidine treated rats regularly produced responses containing a depressor component. The precise pattern of these responses varied between rats. Stimulation with short pulses usually produced an initial pressor response during the period of stimulation which was followed by a secondary longer-lasting depressor phase. With long pulses, there was an initial depressor response during the period of stimulation, followed by a transient rise on cessation of stimulation and then a more slowly recovering depressor phase. The mechanisms involved in the various phases of these responses were analysed by the use of drugs having selective blocking actions.

Tubocurarine and gallamine

A dose of 5 mg/kg of tubocurarine abolished the twitches which accompanied stimulation with both pulse widths. The effects on blood pressure of stimulation with short pulse widths were also completely abolished (Fig. 4). However, in seven experiments, the primary depressor response to stimulation with long pulse widths was not affected or was increased, although the secondary pressor phase and the slow late depressor components were reduced or abolished. In rats treated with guanethidine, but without a noradrenaline infusion, tubocurarine reduced the residual pressor responses during the period of stimulation with long or short pulse widths. In three experiments, gallamine (50 mg/kg) had substantially the same effects as tubocurarine, except that the effect on the secondary depressor response to long pulse width was not as marked.

Hexamethonium

Injections of hexamethonium in doses up to 10 mg/kg had a pressor effect. Responses to spinal stimulation in six experiments with short pulse widths were not affected by hexamethonium. With long pulse widths there was some reduction in the secondary depressor response in two out of nine experiments but no other effect.

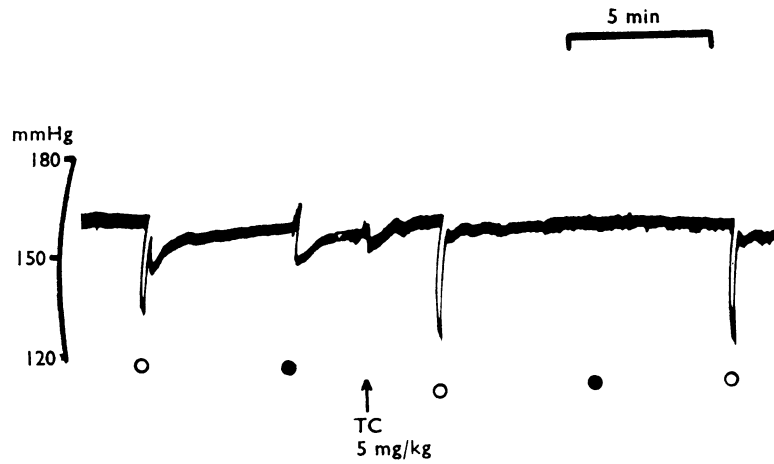

FIG. 4. Effect of tubocurarine (TC) on responses to spinal stimulation with 1 ms (○) and 0·1 ms (●) pulses at 5 Hz for 10 s periods. The rat had been pre-treated with guanethidine (5 mg/kg) and an infusion of (25 μg/kg)/min of noradrenaline was being given. Curvilinear recorder.

Atropine

A dose of 1 mg/kg of atropine abolished the depressor effect of a large dose (5 μg/kg) of acetylcholine and converted it to a pressor effect (Fig. 5). In other experiments it was shown that this dose of atropine abolished the depressor effect of peripheral vagal stimulation. In six experiments the responses to spinal stimulation with short pulse widths were either not affected or biased slightly towards a greater pressor component. With long pulses, the initial depressor response during stimulation was reversed to a pressor response in seven experiments (Fig. 5), but was barely affected in two others (Fig. 8b) even with doses of atropine of up to 5 mg/kg. The secondary slow depressor phase was not affected by atropine.

Neostigmine

A dose of 1 mg/kg of neostigmine in four rats resulted in an increase in the secondary depressor responses to stimulation with long and short pulses, but this effect was abolished by tubocurarine (Fig. 6). In two experiments, neostigmine

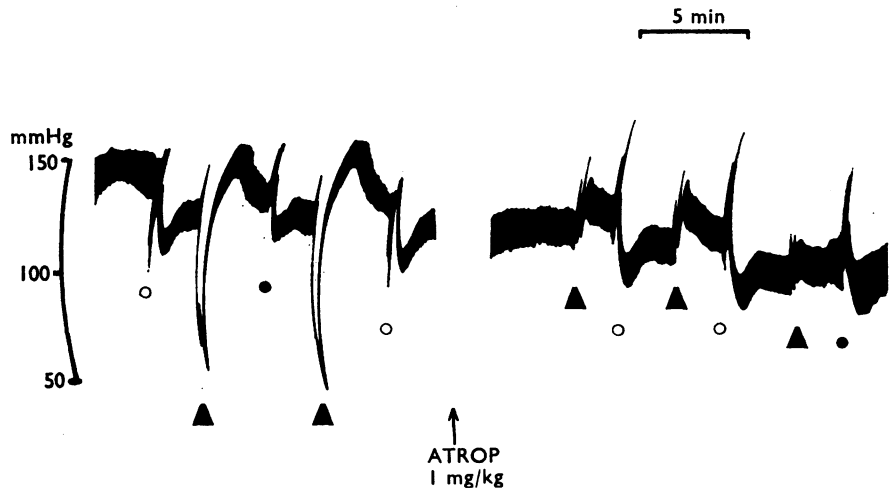

FIG. 5. Effect of atropine (ATROP) in a rat pre-treated with guanethidine (5 mg/kg) and during a noradrenaline infusion of (25 μg/kg)/min on depressor responses to spinal stimulation with pulses of 1 ms (○) and 0·1 ms (●) duration and to injections of 5 μg/kg of acetylcholine (▲). Stimulation was at 5 HZ for 10 s periods. Curvilinear recorder.

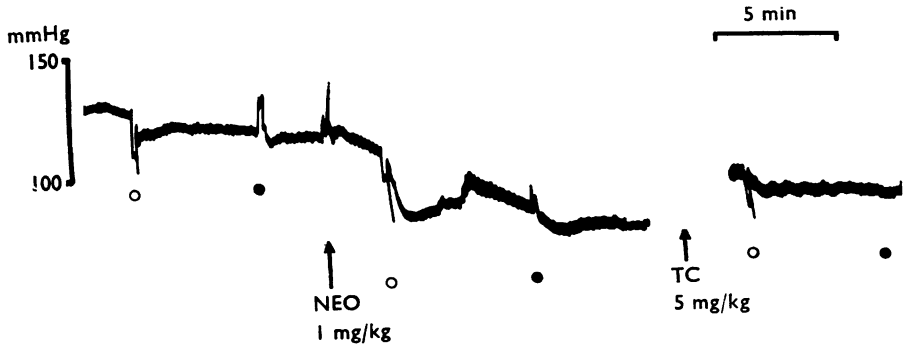

FIG. 6. Effects of neostigmine (NEO) and tubocurarine (TC) on responses to spinal stimulation with 1 ms (○) and 0·1 ms (●) pulses at 5 Hz for 10 s periods. The rat had been pretreated with guanethidine (5 mg/kg) and was infused with noradrenaline (25 μg/kg)/min. Curvilinear recorder.

also caused an increase in the primary depressor phase or converted a primary pressor phase to a depressor phase ; when such an effect occurred it was abolished by atropine (Fig. 7).

Propranolol

In a dose of 1 mg/kg, propranolol produced first a fall in blood pressure followed by a rise to above the previous resting level. In six experiments the initial depressor response during stimulation with long pulses was either markedly reduced, or completely abolished and even reversed into a pressor response (Fig. 8a). This

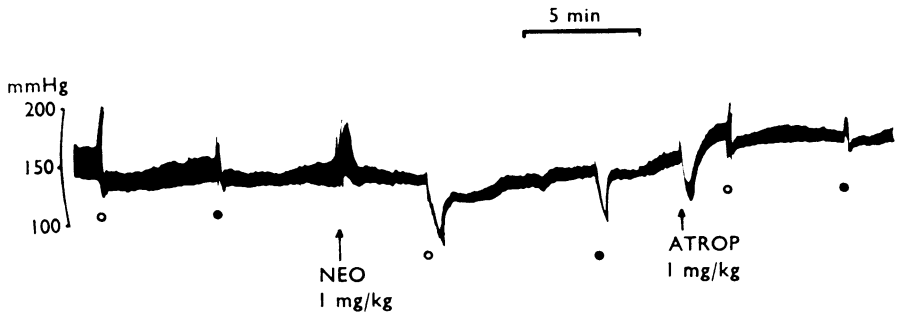

FIG. 7. Effects of neostigmine (NEO) and atropine (ATROP) with 1 ms (○) and 0·1 ms (●) pulses at 5 Hz for 10 s periods. The rat had been pretreated with guanethidine (5 mg/kg) and was infused with noradrenaline (25 μg/kg)/min. Curvilinear recorder.

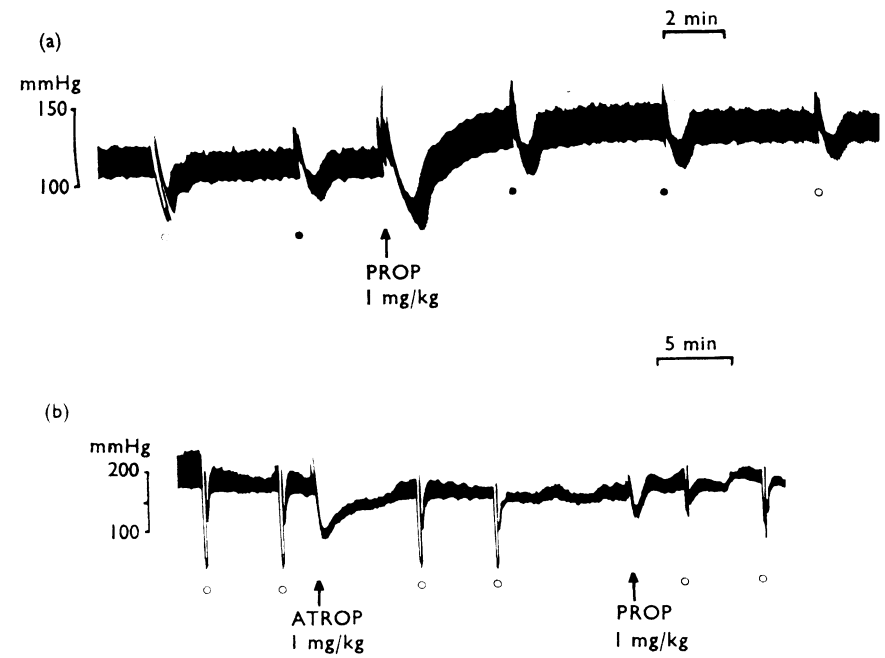

FIG. 8. Effects of propranolol (PROP) in rats pretreated with guanethidine (5 mg/kg) and infused with noradrenaline (25 μg/kg)/min on responses to spinal stimulation with 1 ms (○) and 0·1 ms (●) pulses at 5 Hz for 10 s periods. (a) The depressor response during the period of stimulation with 1 ms pulses was converted to a pressor response. Curvilinear recorder. (b) The marked primary depressor effect was not blocked by atropine (ATROP), but was reduced by propranolol. Rectilinear recorder.

blocking effect of propranolol occurred on depressor responses which were atropine resistant (Fig. 8b). The slow secondary depressor response following stimulation with long pulse widths was slightly reduced after propranolol. The response to stimulation with short pulse widths was not affected.

Dipyridamole

In three experiments, doses of up to 10 mg/kg of dipyridamole had no effect on responses to spinal stimulation.

Adrenalectomy

After adrenalectomy, the responses to spinal stimulation had the same general form as in rats with intact adrenals. However, the depressor response occurring during stimulation with long pulses was not blocked by propranolol, but it was reversed by atropine, as shown in Fig. 9 which illustrates one of three similar experiments.

Discussion

When the blood pressure of the pithed rat was raised by noradrenaline infusion, the response to spinal stimulation was converted from purely pressor to complex containing depressor components. This finding is in contrast to reports by Gillespie & Muir (1967c) that infusion of noradrenaline did not qualitatively alter responses to spinal stimulation. However, their experiments were carried out in rats treated with atropine and tubocurarine and their recording device was not as sensitive to rapid changes in pressure. The depressor responses to spinal stimulation were clearer when the effects of stimulation of noradrenergic sympathetic nerves was abolished by guanethidine.

Stimulation of the thoracico–lumbar region of the spinal cord will excite many types of fibres. Smaller diameter B and C fibres would have a higher pulse-width threshold than the larger diameter myelinated A fibres. In particular, stimulation of large α-motoneurones to skeletal muscle would be expected to occur with short pulse widths which would have little or no effect on autonomic fibres. Guanethidine had no effect on responses to stimulation with pulses of 0·05 ms duration or

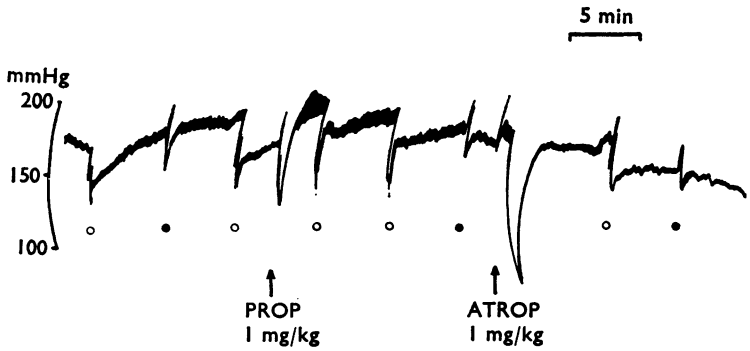

FIG. 9. Adrenalectomized rat pre-treated with guanethidine (5 mg/kg) and infused with noradrenaline (25 μg/kg)/min. Spinal stimulation with 1 ms (○) and 0·1 ms (●) pulses at 5 Hz for 10 s periods. Injections of propranolol (at PROP) and atropine (at ATROP). Curvilinear recorder.

less. Since short pulses do not appear to excite sympathetic nerves, we are puzzled by the use of 0·03 ms pulses by Finch (1971) in experiments to test for changes in responses to sympathetic nerve stimulation in hypertensive rats. The period of stimulation used by Finch was 18 s, whereas we used 10 s, but such a difference has little effect on the response (Gillespie & Muir, 1967a ; G. Dusting, unpublished observations). Tubocurarine and gallamine completely abolished the residual response to stimulation with 0·1 ms pulses in rats pretreated with guanethidine. Since gallamine has no ganglion blocking action and the effect of tubocurarine occurred in the presence of large doses of hexamethonium, the response to stimulation with 0·1 ms pulses can be attributed entirely to stimulation of motoneurones. Stimulation of skeletal muscle could cause an arterial clamping effect resulting in an increase in peripheral resistance and a rise in pressure during the period of stimulation. The increase in the primary pressor response in guanethidine-treated rats with pulse widths above 1 ms could be due to repetitive firing with tetanic contractions elicited by each pulse. The secondary depressor response following stimulation with short pulses is abolished by tubocurarine and can be attributed to a functional or reactive hyperaemia in the stimulated muscles.

Blockade of twitches in response to stimulation of motoneurones with long pulses (1 ms) by tubocurarine and gallamine resulted in reduction of only the secondary prolonged depressor phase of the response that occurred after cessation of stimulation in rats treated with guanethidine and with the blood pressure raised by noradrenaline infusion. Gillespie & Muir (1967a) observed that the pressor response to normal sympathetic stimulation was enhanced by tubocurarine and explained this by facilitation of respiration. However, the pressor response could also be enhanced by the abolition of muscle hyperaemia and this explanation seems more likely since the rats were artificially ventilated with constant output pump and interference with ventilation through stimulation of intercostal or diaphragmatic muscles would be minimal.

The primary depressor phase and the transient pressor phase of the response to stimulation with long pulses in rats given guanethidine and a noradrenaline infusion do not appear to arise from excitation of skeletal muscle. The effects of atropine, although variable, suggest that stimulation of cholinergic fibres is involved, at least in part, in the initial depressor response during stimulation with 1 ms pulses. The enhancement of the primary depressor response by neostigmine and the abolition of this effect by atropine also strongly suggests that cholinergic nerves are being stimulated. This phase of the response was not affected by hexamethonium, therefore it is unlikely to be due to stimulation of preganglionic cholinergic autonomic fibres. It is also unlikely to be due to stimulation of postganglionic autonomic fibres since hexamethonium is as effective as guanethidine in blocking the pressor responses in the normal preparations (Gillespie & Muir, 1967a). It is possible that overflow of acetylcholine from ganglionic synapses and skeletal neuromuscular junctions could result in a sufficient concentration to exert a vasodilator action. The enhancement of the secondary depressor phase of the response to stimulation with long or short pulses by neostigmine may be due to persistence of the vasodilator action of overflow acetylcholine and to a greater functional hyperaemia that could occur from repetitive muscle responses to single pulses in the presence of an anticholinesterase (Blaber & Bowman, 1963) ; these possibilities are supported by the findings that atropine and tubocurarine abolished the effects after they had been enhanced by neostigmine.

Propranolol reduced or abolished the initial depressor response during the period of stimulation with 1 ms pulses and was effective on atropine-resistant depressor responses. This suggests that the effect is mediated by a β-receptor agonist having a vasodilator action. Adrenaline released from the adrenal medulla is a likely candidate since it is a depressor in animals in which the blood pressure has been raised by a noradrenaline infusion (Burn & Rand, 1958). The suggestion is strengthened by the finding that the initial depressor response in adrenalectomized rats was not reduced by propranolol. However, there are two difficulties in attributing the depressor effect to release of adrenaline from the adrenals: first, the time course does not conform to what would be expected, being too rapid in onset and ceasing immediately at the end of stimulation ; secondly, hexamethonium did not have a similar action although it would be expected to block the release of adrenaline caused by stimulation of splanchnic nerves to the medullary cells. The longer lasting secondary depressor phase after stimulation with long pulses was reduced by both propranolol and hexamethonium, suggesting that it was partly due to adrenaline, but it is largely due to muscle hyperaemia. Propranolol often reversed the primary response from depressor to pressor. It has been shown to reduce the hypotensive action of guanethidine (Bein & Brunner, 1966 ; Grewal & Kaul, 1970), but this is due to blockade of the vasodilator action of circulating adrenaline rather than to reversal of the noradrenergic blocking action of guanethidine. Experiments to determine whether propranolol could reverse the noradrenergic neurone blocking effect of guanethidine gave negative results (G. Dusting, unpublished observations).

The bulk of the evidence suggests that the depressor responses to spinal stimulation are due to excitation of motoneurones and the splanchnic nerves to the adrenals, however other possibilities must be considered. Stimulation of dorsal root sensory fibres causes antidromic vasodilatation, at least in skin, and the transmitter involved in this effect is thought to be ATP (Holton, 1959). There is also the possibility of stimulating autonomic nerves in which the transmitter is thought to be ATP (Burnstock, Campbell, Satchell & Smythe, 1970). Depressor responses to ATP are enhanced by 10 mg/kg of dipyridamole (Stafford, 1966 ; Bowman & Stafford, 1968), but the depressor responses to spinal stimulation were not affected by this dose of dipyridamole. This finding suggests that ATP or other depressor adenyl compounds are not mediators of depressor responses to spinal stimulation.

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