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INNERVATION BOTH OF PERI-ORBITAL STRUCTURES AND OF THE HEART BY THE CERVICAL SYMPATHETIC NERVES IN MOUSE, RAT, GUINEA-PIG, RABBIT AND CAT

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1 In anaesthetized rats electrical stimulation of the intact cervical sympathetic nerve produced frequency-dependent lower eyelid contractions and tachycardia.

2 The tachycardia was caused by excitation of efferent fibres since it was equally evident in the pithed rat preparation, and the right nerve was more effective than the left. By contrast, no differences were seen between the responses to right and left vagal stimulation in either rats or rabbits.

3 Guanethidine inhibited both cardiac and eyelid responses, propranolol only the former and phentolamine only the latter, thereby revealing the adrenergic nature of the nerves. Hexamethonium caused partial inhibition and the block was intensified by atropine.

4 The inferior eyelid of mice, guinea-pigs and rabbits as well as the nictitating membrane of rabbits and cats were contracted by cervical sympathetic nerve stimulation. In these species too, tachycardia occurred; this was more pronounced with the right than the left sympathetic nerve. The order of cardiac responsiveness was mouse > rat > guinea-pig > rabbit > cat.

5 In guinea-pigs histamine-induced bronchoconstriction was reduced by cervical sympathetic nerve stimulation.

6 That discrete cardiac pathways exist in the cervical sympathetic nerves is suggested by the reproducibility of the effects within any one species. The accessibility of the nerves greatly simplifies the examination of drugs *in vivo* on two different structures innervated by the sympathetic nervous system.

Introduction

It is generally accepted that the cervical sympathetic nerves convey fibres from the thoracic sympathetic outflow to the superior cervical ganglion from which post-ganglionic neurones predominantly supply structures in the head and neck. Equally the major pre- and post-ganglionic components of the cardiac sympathetic nerves are thought to lie in the chest.

Large (1974) described experiments which showed that stimulation of the cervical sympathetic nerves in rats produced not only retraction of the appropriate eyelid but also marked tachycardia. The results presented here extend the observations in rats and also demonstrate that cardioaccelerator fibres are similarly excited in mice, guinea-pigs, rabbits and cats.

Methods

Experiments in anaesthetized rats

Male Wistar rats, weighing 250-350 g, were anaesthetized with a mixture of chloralose (100-120 mg/kg) and sodium pentobarbitone (30 mg/kg) injected through a nylon cannula terminating in a 17 G needle shaft dwelling in a tail vein.

In some experiments the left iliac, in others the left common carotid artery just below the bifurcation into external and internal branches, was cannulated for blood pressure measurement, the pressure wave being used to trigger a ratemeter. A thread was tied to the right inferior eyelid (or the left in 3 experiments) and connected to a Dynamometer UF1 transducer to record isometric tension changes with an initial tension of 0.4 to 0.5 g. A tracheal cannula was inserted and artificial respiration was maintained with a Miniature Starling pump, rate 72/min and stroke volume 10 ml/kg body weight.

Each vagus in the neck was doubly ligated and sectioned. The cervical sympathetic nerves were left intact and placed on shielded bipolar platinum electrodes immersed in liquid paraffin to avoid current-spread. Each nerve was usually stimulated at a position about 8 mm caudal to the superior

cervical ganglion, although a position 15 to 20 mm was chosen for the left nerve in experiments where a cannula had been placed in the left carotid artery in case the blood vessels supplying the nerve had been damaged. Occasionally the electrode position was altered during an experiment to discover if all the fibres stimulated ran the visible length of the cervical sympathetic nerve. The cathode was caudal to the anode in all but the first 3 experiments when reversal of the polarity was shown not to affect the sizes of the responses. The nerve was stimulated with rectangular pulses, 0.5ms duration, supramaximal voltage (usually 8-10 V) and various frequencies. The frequency-response curves were obtained by initially stimulating at a low frequency, 0.1 or 0.2 Hz, and, after a plateau appeared in the responses, by progressively increasing the frequency to 0.5, 1, 2, 5, 10 and 20 Hz. Similar sequences of stimulation were applied to the cardiac ends of the severed vagi in 5 experiments. All recordings were made on a Devices M-19 recorder.

In a further 6 experiments, following injection of the anaesthetic and after applying artificial respiration, rats were pithed by introducing a steel rod through the left orbit and passing it down the spinal cord. The rod was left *in situ* during the experiment and destruction of the spinal cord was verified by subsequent dissection. In this group of animals the conditions were identical to those previously described with the exception that all blood pressure measurements were made from the carotid artery and the initial eyelid tension was 0.7 to 0.8 g.

The experimental procedures employed in the other species were similar to those in rats with the following exceptions.

Male guinea-pigs, body weight 480-520 g, were anaesthetized with an intraperitoneal injection of chloralose (100 mg/kg) and sodium pentobarbitone (30 mg/kg). The blood pressure was always taken from the left carotid artery, tension changes were measured in the right inferior eyelid only at an initial tension of 1 g and intravenous injections were given through a cannulated left jugular vein. Changes in inflation pressure during artificial respiration were recorded through a pressure transducer connected to the tracheal cannula.

Male mice, Tuck No. 1 strain, weighing 35 to 40 g were used. The most suitable anaesthetic mixture for a stable preparation was found to be chloralose (100-120 mg/kg) and sodium hexobarbitone (100 mg/kg) which was given intravenously through nylon tubing attached to a needle shaft lodged in a tail vein and subsequently used for drug administration. Artificial respiration was given at a rate of 100 strokes/min and the pump set at approximately 1 ml stroke volume, a final adjustment to the latter being made such that the resting blood pressure did not alter by more than 5 mmHg from the level recorded during spontaneous respiration. Following bilateral vagotomy, the right sympathetic nerve only was stimulated. Heart rates were measured by increasing the chart speed during stimulation periods and counting the individual pulses at a time 50 s after the frequencies were changed. Right inferior eyelid contractions were measured from a resting tension of 150-200 mg. Blood pressure was recorded from the left common carotid artery.

Male cats weighing 1.9 to 2.5 kg received chloralose (100 mg/kg) and sodium pentobarbitone (6 mg/kg) intraperitoneally. They were artificially respired at 20 strokes/min with stroke volume 12.5 ml/kg; blood pressure was taken from the left femoral artery and right nictitating membrane contractions were recorded isometrically at a resting tension of 1.5 g. Injections were made through a cannula in the left femoral vein.

Male albino rabbits, weighing 2.5 to 3.0 kg, were anaesthetized with 100 mg/kg chloralose and 30 mg/kg pentobarbitone sodium given via an ear vein; an indwelling needle served for injections given during the experiment. Blood pressure was recorded from the left carotid artery, cannulated near the bifurcation into internal and external carotids. The tension changes were recorded from either the right nictating membrane (initial tension 0.5 g) or the right inferior eyelid (1.5 g). Artificial respiration was given at 40 strokes/min, stroke volume 10 ml/kg body weight.

The following drugs were used: atropine sulphate, guanethidine monosulphate (Ismelin, Ciba Laboratories Ltd), hexamethonium bromide (Koch-Light Laboratories Ltd), histamine acid phosphate (BDH Chemicals Ltd), phentolamine mesylate (Rogitine, Ciba Laboratories Ltd), and (±)-propranolol hydrochloride (Sigma Chemical Company). All doses of drugs are expressed as the salt.

Results

Effects of vagal or sympathetic nerve stimulation in anaesthetized rats

There was no significant difference in the bradycardia evoked by stimulating either the left or right peripheral vagus. Frequency-response curves were virtually superimposable; cardiac arrest occurred in all 5 rats when 50 Hz was applied to the left vagus and in 4 out of 5 rats when the right nerve was stimulated.

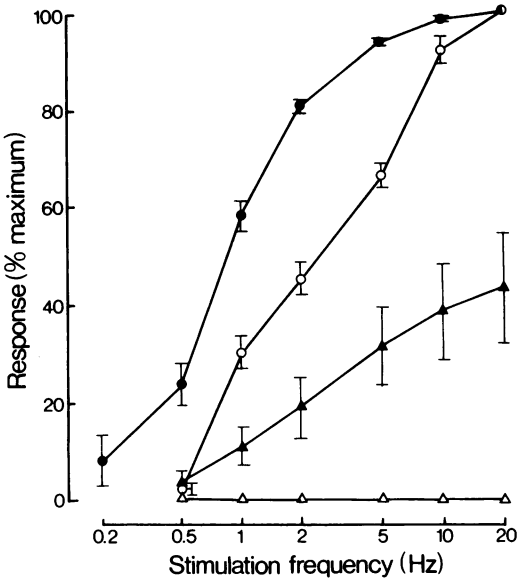

Figure 1 Effects of electrical stimulation of the intact right or left cervical sympathetic nerves on heart rate and on contractions of the right inferior eyelid in anaesthetized rats. Responses are expressed as percentages of the maximal effects caused by stimulation at 20 Hz and are means of determinations in 6 rats; vertical lines show s.e. mean. The nerves were stimulated with rectangular pulses at supramaximal voltage (8-10 V), 0.5 ms pulse width and at progressively increasing frequencies which were changed as soon as a plateau occurred in the responses. Tachycardia on stimulating the right nerve (●) or the left nerve (▲); eyelid contraction with the right nerve (○) or the left nerve (△).

Stimulation of the cervical sympathetic nerve causes retraction of the inferior eyelid (Gertner, 1956). In the present experiments the tension changes in the right eyelid were similar in magnitude when either the intact ipsilateral nerve or its cephalic end after sectioning was stimulated. The minimum effective frequency was usually 1 Hz, maximum tension changes occurred at 10 or 20 Hz and the eyelid was seldom able to maintain its contraction at the latter frequency (Table 1); complete recovery occurred within 60 seconds. The left eyelid tension only was measured in 3 further rats and frequency response curves were found to be virtually identical to those from the right side.

Tachycardia also occurred when the intact right cervical sympathetic nerve was stimulated, an effect which was abolished if the nerve was sectioned caudal to the electrode but which remained unaltered if the nerve was severed cephalad to it. The heart was more sensitive than the eyelid to low frequency stimulation and the tachycardia was nearly maximal with 5 Hz (Table 2). The heart rate often did not recover until 3 to 8 min after ceasing stimulation.

When the left sympathetic nerve was stimulated, frequencies below 1 Hz were ineffective and 20 Hz was needed for maximum tachycardia. The greatest tachycardia measured was invariably smaller than the corresponding response from the right nerve (usually less than 50%) and is illustrated in Figure 1.

The blood pressure records provided a further contrast between the two nerves. When the right one was stimulated mean pressure frequently rose, especially when the tachycardia exceeded 100 beats/min, but pulse pressure rarely changed. By contrast stimulation of the left nerve invariably raised the pulse pressure, occasionally in the absence of tachycardia. In most experiments the pulse pressure was doubled in size when a frequency of 20 Hz was used, but no precise measurements were possible since a slow recording chart speed was employed. Typical responses are illustrated in a later section.

Effects of sympathetic nerve stimulation in pithed rats

When calculated as a percentage of the maximum response to 20 Hz the responses of the right eyelid to nerve stimulation were indistinguishable from those recorded in anaesthetized rats (illustrated in Figure 1). The absolute tension changes were, however, about 30% higher than the previous measurements. These differences may be attributable to the use of a higher initial eyelid tension in the pithed rat preparation.

The cardiovascular changes were similar in most respects to those recorded in the anaesthetized rats except that an increased sensitivity of the heart to low frequency stimulation of the right nerve became apparent (Figure 2). Tachycardia now followed the application of a single shock and 0.1 Hz increased the heart rate by 50 beats/min in 2 experiments. The detailed results are included in Tables 1 and 2.

Effects of sympathetic nerve stimulation in guinea-pigs

The inferior eyelid of the guinea-pig receives an innervation from the cervical sympathetic nerve as does the rat. Contractions of the right eyelid calculated as percentage of the maximum were similar to those in rats when the frequency-response curves were determined. Absolute

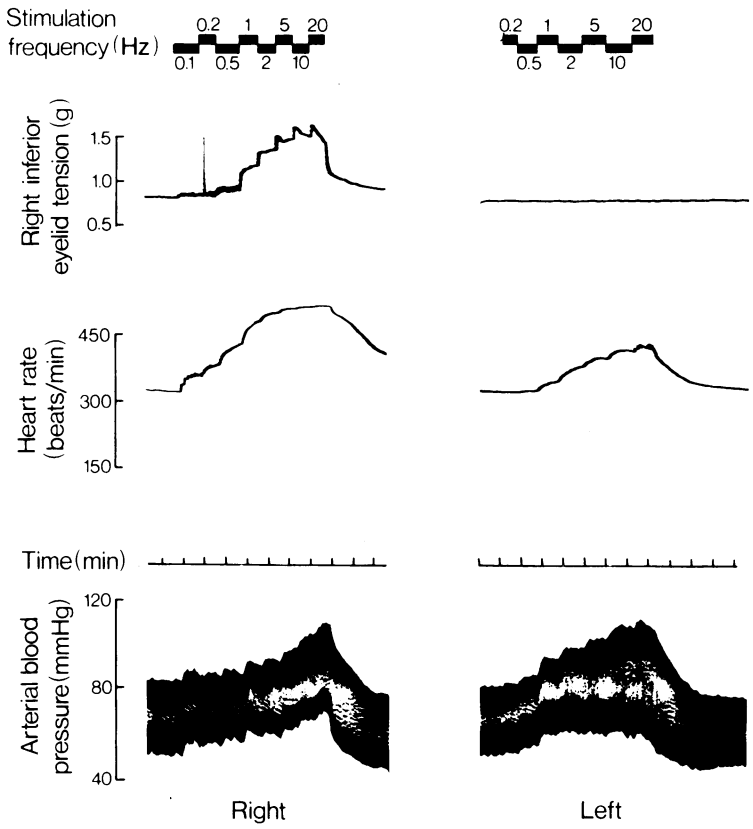

Figure 2 Effects of electrical stimulation of the intact cervical sympathetic nerves in a pithed rat preparation, using progressively increasing frequencies, supramaximal voltage (10 V) and 0.5 ms pulse width. Note particularly that: the eyelid responds only to ipsilateral nerve stimulation; the tachycardia is greater when the nerve on the right is stimulated; and the pulse pressure increases more when the left nerve is stimulated.

Table 1 Isometric tension changes in the right inferior eyelid of anaesthetized mice, rats, guinea-pigs and rabbits, or nictitating membrane of cats and rabbits when the intact right cervical sympathetic nerves were electrically stimulated.

Species	*Mouse*	*Rat*	*Pithed rat*	*Guinea-pig*	*Cat*	*Rabbit*	
No. in group	5	6	6	7	3	2	2
						Eyelid	*Nictitating Membrane*
Initial tension	0.1 – 0.15	0.4 – 0.5	0.7 – 0.8	1.0 – 1.2	1.5	1.0	0.5
Frequency (Hz)							
0.5	0	0	0.08 ± 0.02	0.09 ± 0.02	1.3 ± 0.4	0.1; 0.35	0.1; 0.08
1	0.02 ± 0.01	0.16 ± 0.02	0.23 ± 0.04	0.27 ± 0.03	3.0 ± 0.9	0.48; 0.75	0.16; 0.18
2	0.06 ± 0.03	0.30 ± 0.02	0.38 ± 0.05	0.52 ± 0.03	4.7 ± 1.1	0.72; 1.25	0.20; 0.25
5	0.16 ± 0.02	0.42 ± 0.03	0.56 ± 0.06	0.86 ± 0.05	6.2 ± 1.3	1.08; 1.80	0.35; 0.28
10	0.28 ± 0.04	0.50 ± 0.04	0.67 ± 0.06	1.12 ± 0.06	7.8 ± 1.5	1.40; 2.30	0.40; 0.32
20	0.33 ± 0.02	0.54 ± 0.04	0.72 ± 0.05	1.20 ± 0.08	10.3 ± 1.4	1.20; 2.20	0.30; 0.24

The values are mean tension changes (g) ± s.e. mean except for the results in the rabbit which are from separate experiments.

Table 2 Comparison of tachycardias produced in several species by electrical stimulation of the intact right or left cervical sympathetic nerves.

Nerve	Right							Left				
Species	*Mouse*	*Rat*	*Pithed rat*	*Guinea-pig*	*Rabbit*	*Cat*	*Rat*	*Pithed rat*	*Guinea-pig*	*Rabbit*	*Cat*
No. in group	5	6	6	7	5	3	6	6	7	5	3
Initial heart rate	520 ± 16	299 ± 8	324 ± 4	216 ± 6	300 ± 10	183 ± 7					
Frequency (Hz)											
0.1			23 ± 7								
0.2		13 ± 6	48 ± 10								
0.5	22 ± 2	43 ± 9	82 ± 12	4 ± 1	0	0	5 ± 3	6 ± 5	0		
1	80 ± 7	105 ± 9	125 ± 9	26 ± 3	3 ± 2	2 ± 2	19 ± 7	15 ± 9	3 ± 2	0	0
2	130 ± 13	145 ± 8	143 ± 9	47 ± 5	13 ± 3	5 ± 3	33 ± 12	28 ± 15	7 ± 2	4 ± 2	2 ± 2
5	198 ± 9	168 ± 8	152 ± 10	72 ± 4	32 ± 4	13 ± 9	55 ± 15	39 ± 15	21 ± 5	6 ± 2	3 ± 3
10	223 ± 13	177 ± 7	153 ± 10	91 ± 4	44 ± 5	20 ± 9	68 ± 19	54 ± 19	33 ± 6	14 ± 10	8 ± 4
20	225 ± 14	179 ± 7	153 ± 10	102 ± 4	43 ± 4	22 ± 6	76 ± 20	56 ± 20	39 ± 6	18 ± 8	12 ± 6

Values are mean tachycardia (beats/min) ± s.e. mean.

tensions were different since an initial tension of 1 g was employed and stimulation at 20 Hz produced a mean maximum increase of 1.20 g (Table 1).

When the intact sympathetic nerves were stimulated tachycardia occurred, more so with the right than the left. The absolute change in heart rate was smaller in this species; for instance, when the right nerve was stimulated at 20 Hz a tachycardia of 115 beats/min was never exceeded (Table 2).

In two guinea-pigs resistance of the lungs to inflation was also measured and intravenous histamine was periodically given to increase inflation pressure. Stimulation of the right sympathetic nerve, beginning 60 s prior to histamine injection, caused a frequency-dependent inhibition of the induced bronchoconstriction (about 80% reduction at 20 Hz) which was less pronounced if stimulation began 5 s before the injection. This 'histamine antagonism' may have involved sympathetic bronchodilator fibres in the cervical nerve.

Effects of sympathetic nerve stimulation in mice

The right eyelid of the mouse contracted during cervical nerve stimulation thereby resembling the homologous structures of the other species. The frequency-response curve was flattened at the lower frequencies such that 5 Hz was needed for a 50% maximal response compared with about 2 Hz for a similar effect in rats and guinea-pigs (Table 1).

Stimulation of the right nerve caused tachycardia and the frequency-response curve (% maximum) lay between those constructed for rats and guinea-pigs. When measured in beats/min a greater tachycardia was seen in mice than in any other species, a maximum increase of 250 beats/min occurring in two of the mice when 20 Hz was used (Table 2).

Effects of sympathetic nerve stimulation in cats

Isometric contractions of the right nictitating membrane were recorded to stimulation of the intact cervical nerve; the threshold frequency was 0.5 Hz and larger responses occurred as the frequency was increased to 20 Hz, the highest employed, with which the contraction was well maintained in 2 out of 3 cats (Table 1).

By contrast with the other species the tachycardia to cervical nerve stimulation was small, indeed one cat failed to respond to frequencies lower than 20 Hz, and the left nerve was again less effective than the right (Table 2).

Mean blood pressure increased progressively in each animal as the frequency of stimulation was

raised, the pressor response reaching a maximum of 40 to 60 mmHg. This effect was seen after nerve section only if the caudal ends of the severed nerves were stimulated, and was coincident with the tachycardia. Pulse pressure was rarely affected in these experiments.

Effects of vagal or sympathetic nerve stimulation in rabbits

In a similar manner to rats, the rabbits responded with a frequency-dependent bradycardia when the cardiac end of each sectioned vagus was stimulated and there was no significant difference between right and left vagi.

Fibres in the right sympathetic nerve were found to innervate both the ipsilateral nictitating membrane and inferior eyelid. Two experiments were performed on the membrane and two others on the eyelid instead as a means of establishing that the nerve being stimulated was the cervical sympathetic; since such a limited number of observations was made the results from each experiment are separately recorded in Table 1. Neither the inferior eyelid nor the nictitating membrane of the rabbit was able to maintain the response to 10 Hz stimulation which proved to be a more effective frequency than did 20 Hz.

Cardiovascular changes occurred on stimulating these nerves. The tachycardia never exceeded 65 beats/min and was not seen with frequencies lower than 1 Hz; recovery generally occurred within 60 seconds. Moreover, although stimulation of the left nerve produced slight increases in rate (about 40% of the maximal effect of right sympathetic nerve stimulation) the pulse pressure was mainly unaffected (Table 2). Small increases in blood pressure invariably occurred when each nerve was stimulated but these disappeared if, after nerve section, only the cephalic end was stimulated.

The action of some drugs on responses to sympathetic nerve stimulation

Most of the experiments in this section were performed on rats.

The adrenergic nature of the nerves innervating the heart and the eyelid was confirmed when the adrenergic neurone blocking agent guanethidine (3 mg/kg) reduced or abolished the effects on both structures of intermittent stimulation at 10 Hz, an inhibitory effect which was always preceded by sympathomimesis. Further support was obtained from the effects of α- and β-adrenoceptor blocking drugs, phentolamine (2.5 mg/kg) and propranolol (0.3 mg/kg) respectively. The former prevented the eyelid contraction but had no discernible effect on the tachycardia and the latter selectively abolished the cardiac response whilst slightly increasing the response of the eyelid.

The effects of hexamethonium were less reproducible. At all doses (2.5 to 25 mg/kg) responses to nerve stimulation of both the heart and eyelid were reduced, the latter more than the former. Raising the dose of hexamethonium from 10 mg/kg to 25 mg/kg produced no greater suppression of the tachycardia but the eyelid response was further blocked. In 4 experiments frequency-response curves were determined before and after 5 or 10 mg/kg hexamethonium; the responses to high frequency stimulation of the eyelid were more strongly inhibited than those to low frequency stimulation as is usually the case with ganglion blockade, but curiously the reverse was true with the cardioaccelerator response.

In 3 experiments atropine (1 mg/kg), following hexamethonium administration, further suppressed both cardiac and eyelid responses, the effect on heart rate being the more pronounced. Atropine given alone in two rats did not affect the eyelid responses but slightly reduced the tachycardia when either the right or the left nerve was stimulated at 10 Hz intermittently.

Two experiments were performed in the guinea-pig using hexamethonium 10 mg/kg; the drug depressed both the cardiac and eyelid responses to 10 Hz stimulation, the former by 30 and 50% the latter by 70 and 60% respectively in the two animals. In one of these experiments, the guinea-pig was further given atropine 1 mg/kg and the two drugs in combination almost completely obliterated the responses of both tissues. Four rabbits were given 5 mg/kg hexamethonium, and in each case the response of the eyelid to 10 Hz stimulation was reduced by a greater extent than that of the heart (a mean of 75% compared with 40%). In the one cat which received 5 mg/kg hexamethonium a complete suppression occurred of the tachycardia to 10 Hz stimulation whilst the nictating membrane response was reduced by 75%.

Whenever hexamethonium was given to animals where vasopressor responses were evident there was a dramatic reduction in the pressor effect regardless of the extent of cardiac or eyelid inhibition.

Discussion

Ganglion cells, in numbers running into thousands, are present along the length of the cervical sympathetic trunk and may be scattered or aggregated into visible ganglia (for diagram showing arrangements of pre- and post-ganglionic fibres from studies in cats and rabbits see Douglas, Lywood & Straub, 1960). It might therefore be

anticipated that electrical stimulation of the intact cervical sympathetic nerve should evoke responses not only from structures lying clearly on the nervous pathways cephalad to the electrodes but also from organs more remote which are considered to receive their major innervation from other sources.

In the present study several species responded with cardioacceleration when the cervical sympathetic nerves were stimulated. The reproducibility of the cardiac responses among these animals, with the possible exception of the cat, suggests that it was not a case of aberrant sympathetic fibres being excited but, instead, a well-defined regular nervous pathway. It seems most surprising, in view of the countless investigations of the sympathetic nervous system over recent decades, that such a major pathway has not previously been described.

The first quantitative assessments in this study were made in rats. It soon became evident that tachycardia in excess of 150 beats/min could be regularly evoked with frequencies of 5 or 10 Hz applied either to the intact right sympathetic nerve or to the sectioned caudal end. The participation of afferent fibres in reflex activation of the heart was ruled out by the demonstration that tachycardia still occurred in rats whose spinal cord had been destroyed by orbital pithing.

The frequency-response curve of the heart to stimulation of the right nerve was changed after pithing. A more pronounced tachycardia occurred in the frequency range 0.1 to 0.5 Hz, and indeed responses of 80 and 90% maximal were observed with frequencies of 1 and 2 Hz respectively compared with the 2 and 5 Hz required in anaesthetized rats. It is conceivable that when the central nervous system remained intact either compensatory reflex mechanisms were generated by the cardioacceleration or else afferent fibres with an ultimate modulating function were being directly excited also. Whatever should be the explanation the results in the pithed rat preparation were remarkably similar to the graphs of cardioacceleration caused by stimulation of the spinal cord at the level C7-T1 with a pithing rod (Armstrong & Boura, 1973).

Both the latter authors and Gillespie, Maclaren & Pollock (1970) reported that little change in blood pressure accompanied the cardioacceleration which the localized spinal stimulation evoked. In the present investigations rises in blood pressure were often recorded, especially with the higher frequencies of stimulation. It is unlikely that these responses were simply generated by the primary cardiac effect, particularly since both diastolic and systolic pressures were elevated. Therefore vasoconstrictor fibres seem to exist in this nerve although their regional distribution is unknown.

It was not entirely unexpected that stimulation of the left nerve should produce a smaller tachycardia accompanied by a rise in pulse pressure. Furnival, Linden & Snow (1973) showed that, in dogs, stimulation of the left cardiac sympathetic nerves in the chest caused a smaller chronotropic but a greater inotropic effect than the nerves on the right. A number of references are cited in this paper which provide evidence of an uneven anatomical distribution of the left and right sympathetic fibres. Furthermore, Armstrong & Boura (1970) claimed that stimulation of the left sympathetic nerves of cats caused only positive inotropic effects whilst the nerves on the right produced tachycardia. It is therefore possible that in rats, guinea-pigs and rabbits cardiac efferent fibres running in the right cervical sympathetic nerve predominantly innervate the sino-atrial node whereas a less prominent nodal but a more obvious ventricular innervation is supplied by the left nerve. It should be emphasized that no conclusive evidence is yet available, since, as was indicated by Furnival *et al.* (1973), attempts must be made to control changes in parameters such as heart rate and blood pressure which could indirectly alter the inotropic state of the myocardium.

In a few experiments atropine was administered to discover whether vagal inhibitory fibres might have been simultaneously excited with the sympathetic nerves, thereby reducing the cardioacceleration which would have occurred by solely stimulating the sympathetic supply. This was particularly important during excitation of the left nerve which invariably produced smaller cardiac effects than its right counterpart. Atropine never potentiated the tachycardia, indeed a small reduction in the effect was the rule. This provides further support for a differential distribution of cardiac fibres in each nerve.

There are species differences in the absolute values for maximum tachycardia; in mice the increase of 225 beats/min over resting levels of 520 beats/min was a change of 43%; in anaesthetized rats it was 60%, pithed rats 47%, guinea-pigs 47%, rabbits 14% and in cats 8%. No ready explanation is available for these differences although experiments are planned in which a variety of cardiac stimuli will be compared with the ability of the sympathetic nervous system to evoke responses to discover whether the limitations reside in the nervous system or the effector organ.

Stimulation of the intact cervical sympathetic nerve also resulted in tension changes of the ipsilateral inferior eyelid or the nictitating membrane. The reasonable surmise that mice and guinea-pigs would so respond, despite an absence

of such investigations in the literature, was confirmed. Certain characteristics of response were common to all the species: the threshold frequency was about 1 Hz and maximum increases in tension occurred with 20 Hz; the muscles were seldom able to maintain their tension at this frequency. In none of the experiments did stimulation of the contralateral nerve produce contraction of the eyelid.

Histamine-induced rises in inflation pressure in the guinea-pig were reduced by concurrent stimulation of the right cervical sympathetic nerve. Daly & Mount (1951) reported bronchodilatation in the cat when the caudal end of the cervical sympathetic nerves were stimulated although Petrovskaia (1939) induced bronchoconstriction in guinea-pig isolated lungs with sympathetic stimulation, which was converted to bronchodilatation in the presence of ergotoxine. The present findings might be the result of stimulating sympathetic bronchodilator fibres but since the airway changes were accompanied by tachycardia and rises in blood pressure they could conceivably have been brought about indirectly.

Although there is little doubt that adrenergic nerves are ultimately responsible for both the cardiac and eyelid responses described in this paper the anatomical pathway taken by the cardiac fibres is not clear. Studies with hexamethonium alone and in combination with atropine suggest that the majority, if not all, of the stimulated sympathetic fibres were pre-ganglionic. It would appear reasonable on anatomical grounds that the fibres emerge from the upper thoracic region of the spinal cord and extend cephalad in the cervical nerve. Two explanations, advanced by Daly & Mount (1951) to explain their findings of bronchodilatation in the cat with cervical sympathetic stimulation, provide plausible alternatives for the subsequent pathways. The first assumes that the pre-ganglionic fibres loop near the superior cervical ganglion and then descend the nerve to synapse in, say, the stellate ganglion. The second involves a preganglionic axon reflex whereby fibres normally running up to the superior cervical ganglion branch at a lower level to make additional synaptic contacts with stellate ganglion cells. No evidence appears from the present studies to favour one explanation or the other.

The results in the present paper suggest that a major addition is available to the list of preparations *in vivo* for the study of sympathetically-innervated structures. A very simple dissection technique reveals nerves in the neck of several mammalian species whose stimulation produces effects both on accessible peri-orbital structures and on the heart. Thus the need for thoracotomy to expose paravertebral sympathetic ganglia for electrical stimulation is avoided and the analysis of drugs which modify sympathetic transmission is made relatively simple.

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