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EVIDENCE FOR THE INVOLVEMENT IN THE BARORECEPTOR REFLEX OF A DESCENDING INHIBITORY PATHWAY

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SUMMARY

1. The onset and time course of baroreceptor inhibition of pre- and post-ganglionic sympathetic reflex activity has been examined in the anaesthetized cat.

2. The shortest time to the onset of inhibition of an intercostal evoked reflex response in cardiac and renal nerve was less than 90 msec following a rise in pressure in a carotid sinus blind sac, and around 55 msec following stimulation of the ipsilateral sinus nerve. The cardiac nerve response was completely inhibited before the renal nerve response.

3. Because of the long delays in the somato-sympathetic reflex pathway it is argued that these minimum times will be much less than the real central delay of baroreceptor inhibition. These were estimated by adding on the central times for the somato-sympathetic reflexes to give latencies of 94–143 msec for the inhibition.

4. A spinal sympathetic reflex was inhibited by 30–75% following a rise in pressure in a carotid sinus blind sac or sinus nerve stimulation. The minimum time for this inhibition was around 100 msec.

5. The baroreceptor inhibition of the spinal sympathetic reflex was abolished following section of a restricted region in the dorsolateral part of the lateral funiculus of the cervical spinal cord.

6. Both pre- and post-ganglionic reflexes could be inhibited when stimulating within three regions of the medulla oblongata. The latency to inhibition elicited from the ventromedial reticular formation was short, some 5–30 msec, whereas that elicited from a ventrolateral region or the mid line raphe nucleus was long, some 90–160 msec.

7. The possibility is discussed that the baroreceptor inhibition of both the pre- and post-ganglionic reflexes examined in this study is occurring at the spinal level via a pathway from either the raphe nuclei or ventrolateral medulla.

INTRODUCTION

In recent years a number of investigators have attempted to trace the pathways in the brain stem of the baroreceptor afferents (Humphrey, 1967; Crill & Reis, 1968; Miura & Reis, 1969, 1972; Seller & Illert, 1969; Biscoe & Sampson, 1970*a*, *b*; McAllen & Spyer, 1972). The evidence would suggest that their pathway on to the sympathetic neurones is multisynaptic. In agreement with this is the observation by several groups of workers that the time involved in the inhibitory pathway is remarkably long, 200–300 msec (Kezdi & Geller, 1968; Green & Heffron, 1968; Coote & Downman, 1969), or somewhat less 181 ± 23 msec (Richter, Keck & Seller, 1970). The question arises, has this pathway a long latency because the baroreceptor afferent volley utilizes a slowly conducting descending pathway to the spinal cord, there inhibiting sympathetic neurones? A number of investigators have referred to this possibility but no good experimental evidence has been forthcoming (Kahn & Mills, 1967; Illert & Seller, 1969; Kirchner, Sato & Weidinger, 1971) although recently Gebber, Taylor & Weaver (1973) have provided some evidence that such a pathway must exist. The present experiments were designed to throw some light on this question by examining the onset and time course of baroreceptor inhibition of reflex activity in sympathetic nerves, and comparing this latency with that obtained by electrically stimulating within sympatho-inhibitory regions of the medulla oblongata. Two sympathetic reflex responses were examined, one dependent on pathways long-circuited via the brain stem and the other dependent on spinal segmental pathways alone.

METHODS

Experiments were performed on twenty cats anaesthetized with chloralose (35 mg/kg) and urethane (700 mg/kg) given either intraperitoneally or intravenously after initial halothane induction. Blood pressure was recorded via a polyethylene cannula filled with heparinized 0·9 % saline and inserted towards the heart in the left common carotid artery and connected to a pressure transducer (Bell & Howell). The output was amplified and displayed on a pen recorder (Devices Ltd). The left carotid sinus was prepared by the blind sac technique (Moissejeff, 1927) and intrasinusal pressure was measured via a polyethylene cannula in the external carotid artery connected to a pressure transducer (Southern Electronics). The output of the transducer was fed through a carrier amplifier and displayed on one channel of an oscilloscope (Tektronix 565). The left common carotid artery distal to the blood pressure cannula was cannulated towards the sinus with a polyethylene cannula filled with warm (37° C) 0·9 % NaCl. This was connected to a pressure generating device which enabled a precisely timed pressure wave of predetermined magnitude to distend the sinus. A solenoid tap in this device was controlled by a relay activated from either an S8 stimulator (Grass Instruments Ltd) or from a Digitimer (Devices Ltd).

The inferior cardiac nerve (CN) was exposed retropleurally after removing the heads of the second and third ribs. The renal nerves (RN) were dissected retroperitoneally in the region of the renal artery. Thoracic white rami communicantes (WR) and intercostal nerves (IC) were exposed retropleurally between the ribs. The left sinus nerve was exposed by a lateral approach to the glossopharyngeal nerve in the region below the tympanic bulla after removing the lateral cervical and suprahyoid muscles. Pools were made from skin flaps and all nerves and exposed tissues were covered with liquid paraffin at 37° C. Activity was recorded from the central cut ends of nerves using fine silver wire electrodes and amplified and displayed on an oscilloscope (Tektronix 565). In a number of experiments between ten and twenty reflex responses were averaged for each test using a Physioscope 800 computer (Intertechnique Ltd). Subsequently the size of the reflex responses was determined by measuring their area in projected enlargements of photographic records. Intercostal nerves and the sinus nerve were stimulated via fine silver wire electrodes. Square-wave pulses 0·2 msec in duration were delivered from a Digitimer or an S8 stimulator synchronized with the oscilloscope time base. When stimulating in the brain stem the head was held in a stereotaxic head holder and tilted nose down at 45° to the Horsley–Clarke plane to allow the vertical stimulating electrode to avoid the tentorium cerebelli. The brain stem was stimulated through a unipolar electrode (15–30 kΩ resistance) inserted after removal of the occipital bone and the cerebellum. Square-wave negative going pulses of 1 msec duration and at a frequency of 100 Hz were applied, via an isolation unit. Routinely animals were paralysed with gallamine triethiodide (Flaxedil 4 mg/kg) given I.V. and maintained on artificial respiration. Care was taken to ensure that adequate anaesthesia was maintained throughout the experimental period by allowing the animals to recover from the paralysing drug from time to time, using a sluggish blink reflex and the absence of flexor reflex response as indicators. At the end of some experiments the brain stem or spinal cord was removed and fixed in 10% formal saline; serial frozen sections (50 μm) were cut in the appropriate plane and subsequently stained with luxol fast blue and cresyl violet (Kluver & Barrera, 1953). The site and depth of electrode tracks were located in the brain sections by examination under the microscope and selected sections drawn from projected enlargements. The spinal cord sections were similarly examined.

RESULTS

Effect of raising the pressure in an isolated carotid sinus on the late reflex discharge in cardiac and renal nerves

Maximal reflex responses in the inferior cardiac (CN) and renal nerves (RN) were elicited by single shock stimulation of the central cut end of an ipsilateral intercostal nerve, usually the fifth (IC5). These reflex responses had latencies (60–120 msec) similar to those described by Coote & Downman (1966) for the late discharges in CN and RN, which were shown to be mediated via a pathway passing through the medulla. The following procedure was adopted for testing the effect of carotid sinus distension on these reflex discharges. The patent common carotid artery contralateral to the Moissejeff sac was occluded and when systemic arterial blood pressure was steady five control IC5-CN and IC5-RN reflex

responses were elicited. These were then tested during carotid sinus distension from 0 to 200 mmHg by stimulating the IC5 at a precisely determined time interval following the rise of sinus pressure. The procedure was repeated and the IC5-CN and IC5-RN reflex responses tested at a number of intervals of time following the pressure rise (Fig. 1). In most cases two or more tests were carried out at each interval. The

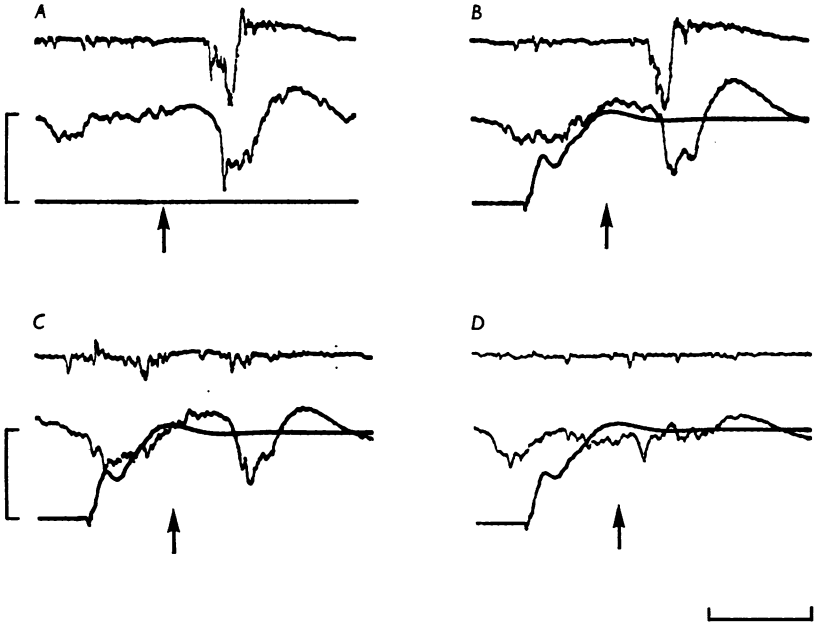

Fig. 1. Inhibitory effects of a rise of carotid sinus pressure on CN and RN reflex responses. *A*, maximal responses in CN (upper trace) and RN (middle trace) elicited by single shock stimulation of IC5. Stimulus at arrow. Lower trace carotid sinus pressure. IC5 stimulated at 119, 125 and 138 msec after the onset of a rise of sinus pressure to 200 mmHg in *B*, *C* and *D* respectively. *B*, both CN and RN reflex responses were present. *C*, RN response was present, whilst CN response was abolished. *D*, both reflex responses were inhibited. Anaesthetized cat. Vertical calibration = 200 mmHg sinus pressure and 150 μV for CN and RN records. Horizontal calibration = 150 msec.

magnitude of the test reflex responses was then expressed as a percentage of the control and plotted against the time interval measured from the beginning of the pressure rise to the reflex stimulus artifact. Maximum inhibition was observed at a mean latency of 196 msec (range 125–330 msec, three cats) for CN and 229 msec (range 138–342 msec, four cats) for RN. This meant that at these and longer intervals the reflex responses could not be elicited. Of particular interest was the finding that in any

one experiment the cardiac nerve reflex response was abolished at a shorter time after the sinus pressure rise than the renal nerve reflex response (Fig. 1). In the three cats the difference was 13 msec in one, 19 msec in another, and 12 msec in the third. The time course of the inhibition in one experiment is illustrated in the graph in Fig. 2. It can be seen from this that the point at which some inhibitory effects can first be seen is considerably earlier than the point of complete inhibition, it being less than 90 msec after the sinus pressure rise in this example.

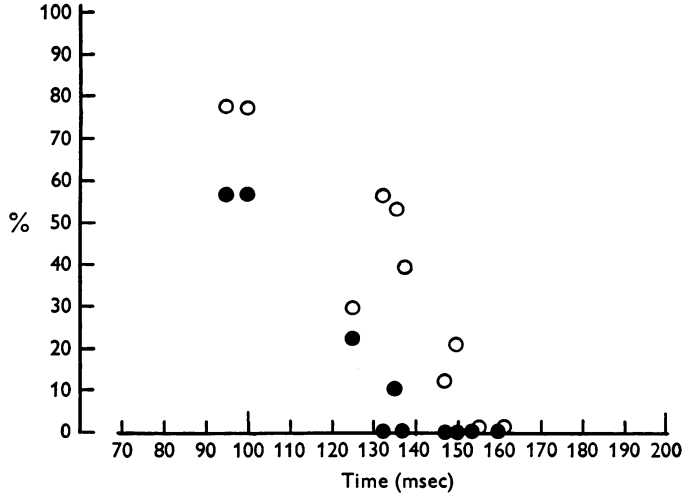

Fig. 2. The degree of inhibition of IC-CN and IC-RN reflex responses when elicited at various times after an increase of carotid sinus pressure to 200 mmHg. The average size of three CN (filled circles) and three RN (open circles) maximal reflex responses expressed as a percentage of the average of three control responses (ordinate) is plotted for the time intervals between the onset of the rise of sinus pressure and IC5 stimulation (abscissa). Anaesthetized cat. Mean IC5-CN reflex latency = 70 msec. Mean IC5-RN reflex latency = 98 msec.

The CN and RN reflex discharges were tested during pressure increases of different magnitude. Such experiments showed that the latency to maximum inhibition increased as the magnitude of the pressure change decreased. For example in one experiment, during a rise in sinus pressure of 200 mmHg, the time to complete inhibition for CN was 125 msec and for RN was 138 msec. This latency increased with lower pressure stimuli. For a rise in sinus pressure of 94 mmHg it reached a maximum of 180 msec for complete inhibition of CN and 207 msec for RN. Thereafter, complete inhibition of the reflex response was not observed.

The effect of raising the pressure in an isolated carotid sinus on a spinal sympathetic reflex

In two cats a spinal sympathetic reflex recorded in the tenth or eleventh thoracic white ramus was tested during distension to 200 mmHg, of a unilateral carotid sinus blind sac. A similar procedure to that described above was adopted. With both carotid arteries occluded twenty IC evoked sympathetic reflex responses, elicited at 5 or 10 sec intervals, were averaged by the computer. These were compared with twenty averaged responses evoked after carotid sinus distension. Examples of responses averaged in this manner are shown in Fig. 3 in which the

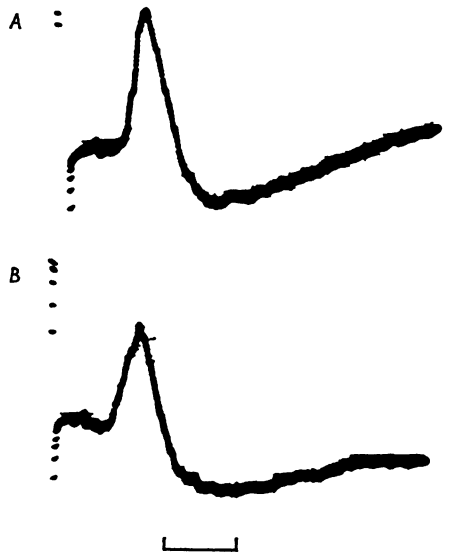

Fig. 3. Effect of carotid sinus distension on IC-WR spinal reflex response. Records show summed responses from an average response computer. *A*, the summed response of twenty maximal T11WR reflex responses elicited by a single stimulus to IC10 at 5 sec intervals. Stimulus artifact at beginning of trace. *B*, average of 20 T11WR responses elicited by IC10 stimulation 125 msec after the onset of an increase of sinus pressure to 200 mmHg. Anaesthetized cat. Time calibration = 9·6 msec. Mean IC10-T11WR reflex latency = 7·5 msec.

IC10-T11WR reflex responses in *B* were tested 125 msec after carotid sinus distension and showed some 30% depression. This procedure was repeated at a number of intervals between the pressure ramp and the elicitation of the WR response. The size of the averaged responses expressed as a percentage of the average of twenty control responses

was plotted against the time interval after the rise of pressure inside the sinus. Fig. 4 shows such a graph from one experiment. The WR response was depressed by some 30% at 125 msec after the sinus pressure rise and by 20% at 250 msec. At 1 sec after the sinus pressure rise, the WR reflex response had recovered almost to control size. No attempt was made to determine the onset of inhibition more precisely in these experiments. This was carried out in subsequent experiments in which the reflex responses were examined following electrical stimulation of the sinus nerve.

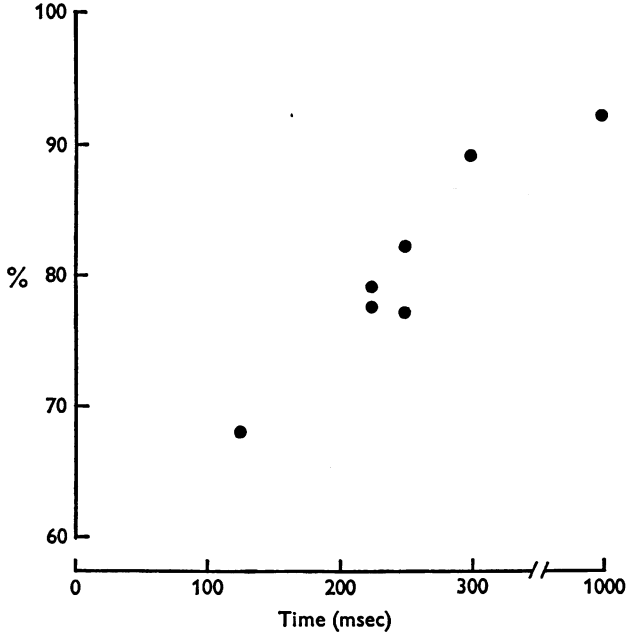

Fig. 4. The time course of the inhibition of a WR spinal reflex response following an increase of carotid sinus pressure. The size of the summed response of twenty maximal T11WR reflex responses elicited by single shock stimulation of IC10 during carotid sinus distension is expressed as a percentage of twenty summed control T11WR responses (ordinate). The time interval between the onset of the rise of sinus pressure and IC10 stimulation for each test is plotted along the abscissa. Anaesthetized cat. Mean IC10-T11WR reflex latency = 7·5 msec.

The effect of electrical stimulation of one sinus nerve on the late reflex discharge in cardiac and renal nerves

Maximal IC-CN and IC-RN reflex responses were examined at different intervals of time following a brief train of stimuli to the sinus nerve. At the commencement of an experiment the sinus nerve was stimulated

with a 5 sec train of stimuli at 100/sec with pulses of 0·2 msec duration, and its effect on blood pressure observed. The stimulus strength was then adjusted to obtain a maximal depressor response and the train length was reduced to give three pulses and the IC-CN and IC-RN reflex responses tested at a number of intervals of time after this brief train. For each interval tested twenty control reflexes were elicited, one every 10 sec, these were averaged and compared to the average of twenty test

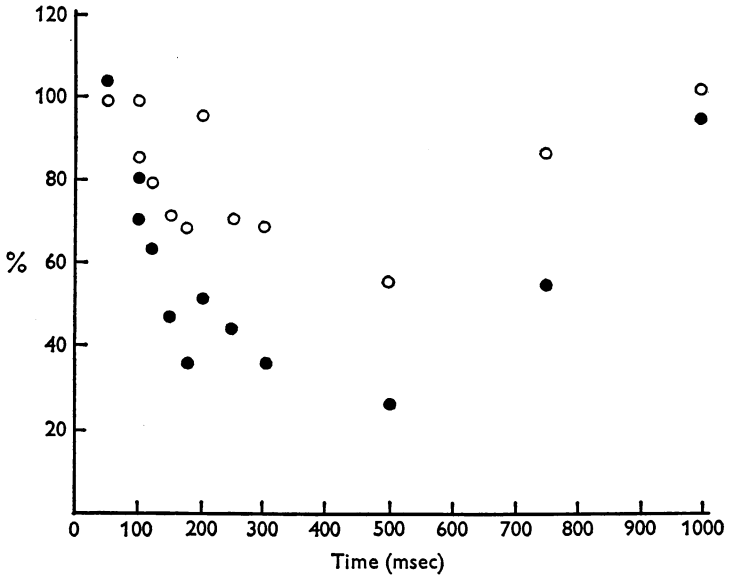

Fig. 5. Effect of sinus nerve stimulation on IC and WR spinal reflex responses. The size of the summed responses of twenty maximal IC9-IC10 (open circles) and IC9-T10WR (filled circles) reflex responses elicited after sinus nerve stimulation (3 shocks, 0·2 msec, 100 Hz) is expressed as a percentage of twenty control reflex responses (ordinate). These values are plotted against the time interval between the first shock to the sinus nerve and IC9 stimulus for each test (abscissa). Anaesthetized cat. Mean IC9-IC10 reflex latency = 4·8 msec. Mean IC9-T10WR reflex latency = 8·0 msec.

reflex responses following sinus nerve stimulation. The intervals were measured from the first stimulus to the sinus nerve to that to the intercostal nerve. Subsequently the size of the averaged test responses expressed as a percentage of the control reflex responses was plotted against the time intervals. Initially the CN response was again reduced more than the RN response. In one experiment, at 55 msec, the IC-CN response was reduced by 83% and the IC-RN response by a little over 35%.

By 90 msec the IC-CN response was depressed by 95% and the IC-RN response by 82%.

The effect of stimulating one carotid sinus nerve on spinal sympathetic reflex response recorded in thoracic white rami

Maximal IC9-T10WR or IC10-T11WR reflex responses were tested before and following sinus nerve stimulation, the procedure being similar to that described in the previous section. Twenty control reflex responses elicited one every 5 sec were averaged and then this was repeated following sinus nerve stimulation. Subsequently the size of the averaged test responses was expressed as a percentage of the previously averaged twenty control responses and then plotted against the intervals of time after the sinus nerve stimulation. An example of such a plot for one experiment is shown in Fig. 5. In this experiment the IC9-T10WR response was depressed by 20% at 100 msec and by a maximum of 75% at 500 msec, it then returned gradually to control size at 1 sec. Similar results were obtained in two other cats although in one animal the onset of inhibition was earlier, there being a 20% reduction evident at 70 msec after the sinus nerve stimulation.

The effect of baroreceptor excitation on a spinal somatic reflex recorded in an intercostal nerve

Maximal IC9-IC10, IC10-IC11 reflex responses were examined simultaneously with and in a similar way to that described for the spinal sympathetic reflex responses. The IC-IC reflex responses were depressed following sinus nerve stimulation but less so than were the sympathetic reflex responses. The time course of the inhibitory effect was, however, very similar. An example is shown in Fig. 5 in which there was some depression of the IC-IC reflex response at 100 msec and a maximum of 45% depression at 500 msec, the IC-IC reflex thereafter increasing in size to reach control size at 1 sec. The commencement of the inhibition was not determined precisely since no intervals were tested between 50 and 100 msec, but since the degree of depression was small at 100 msec the latency was unlikely to have been much less than this.

In each of the above series of experiments the brief train of stimuli to the sinus nerve or carotid sinus distension repeated every 5 or 10 sec caused small falls in blood pressure which were maintained for a series in which twenty reflex responses were tested and averaged. These falls in blood pressure were 10 mmHg or less, probably because the stimulus, whether shocks to the sinus nerve or pressure ramp to a blind sac, was only brief. Such blood pressure changes were unlikely to be affecting

the reflex responses because they were still present at intervals at which the reflex discharges were unaffected.

The effect of section of pathways in the spinal cord

The previous experiments have shown that a spinal sympathetic reflex can be inhibited by baroreceptor activation. The following experiments were performed to determine which region of the spinal cord was important in the mediation of the reflex inhibition. A conditioning testing procedure was conducted as described previously using a sinus nerve conditioning volley. The interval for maximum inhibition of an IC9-T10WR reflex response was then determined. A contralateral hemisection at the seventh cervical level was then performed on the previously exposed spinal cord. The averaged control IC9-T10WR responses and the degree of inhibition of this reflex by sinus nerve stimulation were unaffected by this procedure. Subsequently small cuts were made in the dorsal and lateral funiculi of the seventh cervical segment ipsilateral to the recording site. In two cats it was found that the inhibition of the IC9-T10WR response elicited by sinus nerve stimulation was abolished following a section in the dorsolateral funiculus the position of which was confirmed on subsequent histological examination and is illustrated for one cat in Fig. 6. This section also resulted in a small increase in the size of the sympathetic reflex which was of the order of 10%.

Unfortunately a lesion in this region of the spinal cord also cuts the ascending pathway of the long-circuited sympathetic reflex (Coote & Downman, 1966) and so it was not possible in the above experiments to test the baroreceptor inhibition of this response in the same way.

Timing of inhibition elicited from the brain stem

In six cats regions in the lower brain stem were explored with stimulating electrodes using a ten second train of negative going pulses, 0·2 msec in duration, 3–6 V in strength and at 100 Hz. The effect on blood pressure and spontaneous sympathetic activity was recorded. Having established that a point was inhibitory, i.e. it produced a fall in blood pressure and reduced sympathetic activity, the procedure for measuring the latency of the inhibitory pathway was commenced as follows. Twenty sympathetic reflex responses, one elicited every ten seconds, were averaged. The effect of preceding the reflexes by four negative going pulses at 100 Hz to the inhibitory point in the brain stem was then examined. Twenty responses tested in this manner were averaged and the procedure repeated for a number of intervals between brain stimulus and reflex stimulus. A graph was plotted showing the size of the averaged reflex responses expressed as a percentage of the control reflex responses at a number

of intervals following the brain stimulus in order to determine the minimum time to inhibition. Measurements were made in three inhibitory regions; the ventromedial reticular formation at the level of the obex (Coote, 1964; Scherrer, 1966; Coote & Downman, 1969; Gootman & Cohen, 1971; Kirchner *et al.* 1971); a region close to the mid line in the caudal

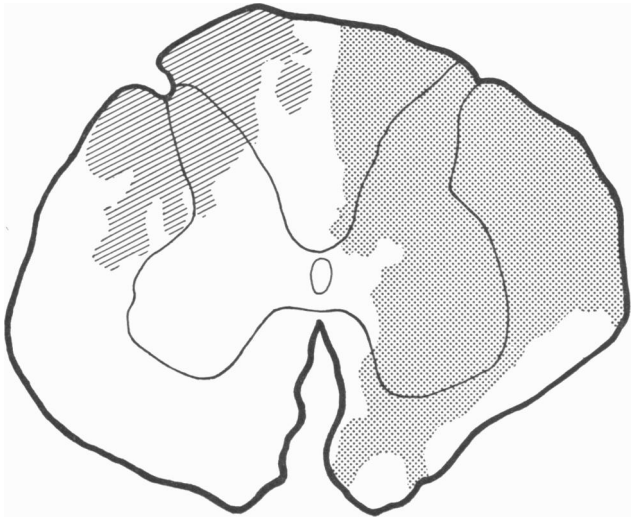

Fig. 6. Diagram of transverse section of spinal cord (C7). The shaded areas represent a superimposed picture of the extent of tissue damage made by knife cuts drawn from serial sections of spinal cord. On the right-hand side of the diagram the shading shows the extent of a hemi-section. On the left, the shaded area indicates the extent of the section through the dorsal and dorsolateral funiculi which abolished the inhibition of the IC9-T10WR spinal reflex response caused by sinus nerve stimulation.

nuclei of the raphe, and a region in the ventrolateral border of the medulla oblongata (Macleod, 1972; Coote & Macleod, 1972). The late reflex response in the renal nerve, which depends on a pathway long-circuited via the brain stem, was first examined. In three cats it was found when stimulating in the ventromedial reticular formation that this sympathetic reflex could be inhibited even when the brain stimulus was commenced after the intercostal nerve stimulus. Thus for an IC5-RN reflex response with an over-all latency of 95 msec the brain stimulus could still inhibit the reflex up to 50 msec after the intercostal stimulus (Fig. 7). This inhibitory pathway, therefore, appears to be a relatively fast one. The time involved in the inhibitory pathway was then calculated assuming that the inhibition was occurring at the spinal segmental level

and not on the afferent relay neurones in the brain stem. There is some justification for this assumption since there is evidence that inhibition elicited from the ventromedial reticular formation exerts its effect on the spinal motor neurone pools (cf. Coote & Downman, 1969). To determine

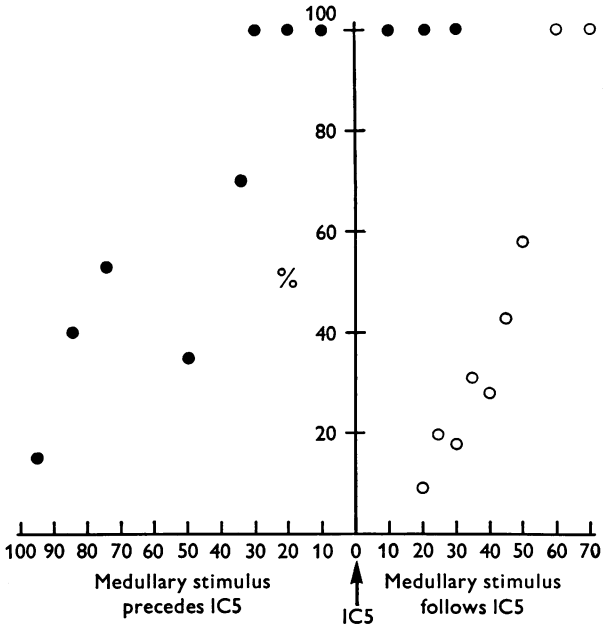

Fig. 7. Time course of the inhibitory effects of electrical stimulation within two areas of the medulla oblongata on IC5-RN reflex responses. The time between the first shock of a short train of stimuli to the medulla (3 shocks, 0·2 msec, 100 Hz) and a single shock to IC5 (T = 0) is shown on the abscissa. The size of the summed response of twenty maximal RN reflex responses tested with brain stimulation at 10 sec intervals expressed as a percentage of twenty control responses is shown for stimulation of ventromedial reticular formation and ventrolateral medulla at various times relative to IC5 stimulation. Open circles, the effects of stimulating a point in the ventromedial reticular formation, 1·0 mm rostral to obex, 1·5 mm lateral to mid line and 3·0 mm depth. Filled circles, the effects of stimulating a point in the ventrolateral medulla, 1·0 mm rostral to obex, 4·0 mm lateral to mid line and 5·0 mm depth. Anaesthetized cat. Mean RN reflex latency = 90 msec.

the time for the inhibitory pathway, the interval between the brain stimulus and the reflex response was estimated and the time involved in conduction along the peripheral efferent pathway subtracted. This latter time was determined by stimulating the T10 ventral root, the outflow for the reflex response in the renal nerve having previously been

limited to the T10 segment by section of the sympathetic chain between T9-T10 and the spinal cord between T10-T11. Thus in the above example the reflex time was 95 msec and the ventral root conduction time was 40 msec leaving a central delay of 55 msec. The latency of inhibition was, therefore, the central delay 55 msec minus 50 msec, the interval between the stimulus to IC5 and the stimulus to the brain, which gives a figure of 5 msec. Different latencies were obtained in other experiments, the range for all cats being 5–30 msec.

In striking contrast to this, in the same animals stimulation within the ventrolateral region of the medulla and the caudal nuclei of the raphe elicited inhibition which had a long latency. For example, when stimulating in the ventrolateral region in the above animal, inhibition began at 35 msec before the intercostal nerve stimulation and was not maximal until 95 msec (Fig. 7). Similarly, an inhibitory point in the mid line nuclei of the caudal medulla had to be stimulated 85 msec before the intercostal nerve in order to maximally inhibit the RN reflex. After adding on the reflex time (95 msec) and subtracting the ventral root time (40 msec) the latency for these inhibitory pathways was estimated to be 90–150 msec for the former and 90–140 msec for the latter region.

A similar series of experiments was carried out in a further three cats to measure the latency of brain stem elicited inhibition of a spinal sympathetic reflex. Latency was measured from the first conditioning stimulus in the brain stem to the intercostal nerve stimulus. A maximal IC9-T10WR reflex response was examined. The minimum latency of inhibition elicited from a point in the ventromedial reticular formation had a range of 10–30 msec, that is, rather similar to that calculated for the effect on the long-circuited sympathetic reflex. Again in contrast, both an inhibitory point in the ventrolateral region of the medulla and in the mid line had a much longer latency. Inhibition began at an interval of 100 msec and was maximum at 160 msec.

It is also particularly interesting that the latency to inhibition elicited from the ventrolateral and mid line regions of the medulla was more similar to the latency of baroreceptor inhibition than was the effect from the ventromedial reticular formation.

DISCUSSION

The present experiments were designed to give information on the length of the central nervous pathway mediating baroreceptor inhibition of sympathetic activity and also to help in determining the site at which this inhibition occurs. We chose to examine sympathetic reflex activity because this enabled a more precise measurement of the time course of

the inhibitory effect elicited by stimulation of the baroreceptor afferent endings. However, such measurement of latencies suffers from a number of disadvantages, notably that in the case of the late large amplitude reflex recorded in post-ganglionic sympathetic nerves to heart and kidney there is a great deal of time involved in the reflex pathway. Therefore, the figures for latency of baroreceptor inhibition are likely to be much longer than given because the measurements were made between the stimulus to the baroreceptors and the stimulus to the somatic nerve eliciting the sympathetic reflex, and thus do not take into account the central delay of this reflex. The reflex time cannot simply be added on because a proportion of this latency is due to conduction along the peripheral efferent pathway. The central reflex time was only determined in a few experiments but if we were to use the central reflex times obtained in a previous study (Coote, 1964; Coote & Downman, 1966) the central time for baroreceptor inhibition would increase by 39–45 msec for cardiac nerve and by 45–53 msec for renal nerve which would give latencies for the inhibition of 94–143 msec. Of course this then would assume that the baroreceptor inhibition was occurring at the spinal level since the central reflex times are for the central nervous pathway on to the final motor neurone at the segmental level. Even without making such a calculation it is evident that the latency of baroreceptor inhibition of vasomotor reflex activity is very long, the minimum figures obtained being 55–90 msec. Very little of this time can be taken up in generating the volley and conduction in the afferent nerve fibres to the central nervous system since this is likely to be quite rapid (Humphrey, 1967; Kezdi & Geller, 1968). It was, therefore, extremely interesting that a spinal sympathetic reflex also could be inhibited by baroreceptor activation and that the latency of this inhibition was also long, at least 70–100 msec, and therefore in the same range as that for the long-circuited reflex in the renal nerve. This of course does not include the central times of the reflex responses. However, since these are short, some 5–10 msec, taking account of them would make little difference to the argument.

The spinal segmental nature of the early reflex response recorded in thoracic or lumbar white rami is now well established (Beacham & Perl, 1964; Coote, 1964; Coote & Downman, 1965; Sato, Tsushima & Fujimori, 1965; Coote, Downman & Weber, 1969). However, the functional significance of such reflex discharges is difficult to assess, unlike that of the post-ganglionic pathways used in this study. Undoubtedly they may contain a component which is cardiomotor or vasomotor but the size of this will probably vary at different levels (Seller, 1972). Coote & Downman (1965) concluded that only a small contribution was made to vasomotor activity by the spinal preganglionic reflex response. This was

based on a comparison of the properties of the early spinal sympathetic reflex recorded in post-ganglionic nerves to heart and kidney (Coote, 1964; Coote & Downman, 1966; Kirchner *et al.* 1971) with the spinal sympathetic reflex so easily elicited in preganglionic thoracic and lumbar white rami (Coote & Downman, 1965). Amongst several others, one of the differences was that the spinal reflex in a thoracic white ramus was unaffected by baroreceptor activation (Coote & Downman, 1965; Coote *et al.* 1969). In contrast in the present investigation we have been able to demonstrate that the baroreceptors have a profound effect on spinal sympathetic reflexes. We have evidence that the size of this inhibitory effect is different at each segmental level and it may have been this together with the techniques used previously that resulted in inhibition being missed. This does not lead the present authors to challenge the conclusion of Coote & Downman (1965) or Coote *et al.* (1969) since the other differences between the major part of the spinal reflex in thoracic white rami and the reflex activity in post-ganglionic nerves to heart and kidney, noted by these authors, still stand. That the baroreceptors may have an effect on sympathetic neurones at the spinal level is evident also from the records of Kirchner *et al.* (1971). Their records show that the spinal reflex recorded in a post-ganglionic renal nerve was occasionally depressed by an increase in blood pressure produced by intravenous noradrenaline. However, probably because of the variability of their results these authors do not come to a clear conclusion about the spinal effects of the baroreceptor inhibition.

The similarity of the time for baroreceptor inhibition of a long-circuited and a spinal sympathetic reflex may well be suggestive of a descending bulbospinal pathway playing a major role in baroreceptor inhibitory effects on both reflex responses as is illustrated in the diagram in Fig. 8. Also suggestive of this are the results from experiments utilizing two outflows, one to heart and the other to kidney. The cardiac nerve reflex response was usually completely inhibited prior to the renal nerve reflex response whose outflow is further away from the brain stem.

In addition, although there is evidence that the baroreceptor afferent fibres can exert an effect on neurones within the hypothalamus (Hilton & Spyer, 1971) and on respiratory neurones within the brain stem (Biscoe & Sampson, 1970*c*), it is interesting that there is a complete absence of evidence in the literature that the baroreceptor reflex exerts its sympatho-inhibitory effect at the brain stem level. If the inhibitory effect is occurring here it is difficult to explain why the latency of the baroreceptor reflex is so long. There is no reason to suppose that the pathway is so multi-synaptic or the transiting afferents so small as to account for the long conduction time, particularly since there is evidence that the baroreceptors

can have an inhibitory effect on some systems in quite a short time. Biscoe & Sampson (1970*c*) described inhibitory effects on phrenic motoneurones elicited by carotid sinus distension or sinus nerve stimulation, with a latency of only 10 msec. They presented evidence suggesting that this inhibition was not occurring at the phrenic motoneurones in the cervical spinal cord but at some point on the respiratory drive pathway, probably in the brain stem.

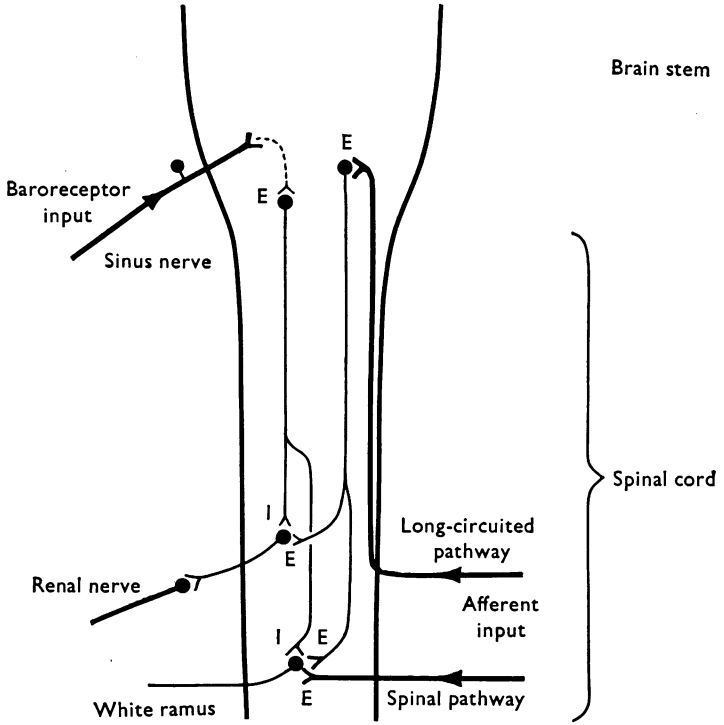

Fig. 8. Diagrammatic representation of the baroreceptor reflex pathway suggested by the results of the present experiments in which afferent and efferent pathways of the same side were investigated. To simplify the diagram these are illustrated bilaterally. I = Inhibition. E = Excitation.

In the present experiments a respiratory muscle reflex, the intercostal reflex, was also shown to be inhibited at a similar latency to the sympathetic reflexes. If this intercostal reflex was being affected in the same way as the phrenic motoneurones in the experiments of Biscoe & Sampson (1970*c*), then the latency to inhibition would have been similarly short. Since it was very long and, therefore, dissimilar, it again seems reasonable to suppose that this inhibition is occurring at the spinal level and the latency is long because it is mediated by a slowly conducting descending

pathway (Fig. 8). Thus, as has been indicated by the recent work of Gebber *et al.* (1973), the baroreceptor reflex inhibits sympathetic reflex responses at the spinal level. Whether other regions of the central nervous system are involved remains to be established.

Three possible pathways arising in the brain stem have been described over which the baroreceptor inhibitory effect could be exerted (Macleod, 1972). One is a reticulo-spinal pathway emanating from the ventromedial reticular formation from which inhibition of sympathetic activity can be obtained (Wang & Brown, 1956; Prout, Coote & Downman, 1964; Kahn & Mills, 1967; Coote & Downman, 1969; Coote *et al.* 1969; Gootman & Cohen, 1971). This pathway is not a likely candidate since in the present experiments the inhibition elicited from this region had a very short latency of 5–30 msec. Therefore, this pathway is very fast, confirming the observations of Gootman & Cohen (1971) who determined the latency to inhibition of spontaneous activity in the splanchnic nerve elicited from the ventromedial reticular formation to be 20–40 msec. In contrast, in the present investigations the minimum latency to baroreceptor inhibition of sympathetic reflex activity is longer. In fact when this is calculated in the same way as was that for the above example, by determining the interval between the conditioning stimulus and the reflex potential in the sympathetic nerve and then subtracting the time measured in the peripheral efferent pathway it is considerably longer, being some 94–143 msec. It seems, therefore, unlikely that the ventromedial region of the brain stem is involved. Such an argument is strengthened by the findings of Humphrey (1967) who was able to evoke activity in this region following baroreceptor stimulation with a latency as small as 10 msec. The other two regions, one in the ventrolateral border of the medulla oblongata, and the second in the caudal nucleus of the raphe, are much more interesting candidates for mediating baroreceptor inhibition. Stimulation in either region elicited an inhibition of both spinal and long-circuited sympathetic reflexes, which was of long latency and similar to the baroreceptor latency. At first sight this latency appears to be markedly different for the two somato-sympathetic reflex responses. However, again calculated as the time between the conditioning stimulus and the reflex bears response, less the time involved in the peripheral efferent pathway, the latency of 90–150 msec and 90–140 msec for the inhibition of the long-circuited reflex bears comparison with the 100–160 msec obtained for the latency to maximum inhibition of the spinal reflex.

Both ventrolateral and mid line regions contain cell bodies whose axons descend in the spinal cord and terminate around sympathetic neurones in the intermediolateral cell column (Dahlstrom & Fuxe, 1965). Evidence presented in the previous paper (Coote & Macleod, 1974)

suggests that these descending systems are inhibitory and conduction in them is slow. This could well account for the time to sympathetic inhibition measured following stimulation within these brain regions. There is, therefore, no need to postulate some complicated multisynaptic pathway arising from them which may eventually involve the fast ventromedial pathway.

Other evidence also suggests that it is one of these slow bulbospinal pathways that is involved in the baroreceptor inhibition. A cut made in the region of the spinal cord referred to earlier, through which the above pathways descend (Macleod, 1972; Coote & Macleod, 1974) was shown in the present experiments to abolish the sinus nerve elicited inhibition of the spinal sympathetic reflex. Such a pathway would account for the long latency of baroreceptor inhibition. It would also help explain the lack of evidence for pulse modulated vasomotor units in the medulla oblongata. This has been somewhat surprising in view of the classical idea of a vasomotor centre in the medulla and also since there is a remarkably close relationship between baroreceptor input and peripheral vasomotor nerve activity (Kezdi & Geller, 1968; Green & Heffron, 1968).

Such a finding is no longer puzzling if one postulates that the pulse modulation is occurring at the spinal vasomotor neurone. The demonstration in the present experiments of a descending bulbospinal baroreceptor pathway makes this latter suggestion a likely possibility.

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