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BARORECEPTOR INPUTS TO THE NUCLEUS TRACTUS SOLITARIUS IN THE CAT: POSTSYNAPTIC ACTIONS AND THE INFLUENCE OF RESPIRATION

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SUMMARY

1. The postsynaptic action of carotid sinus nerve (SN), aortic nerve (AN), superior laryngeal nerve (SLN) and vagal nerve (VN) stimulation has been studied on neurones in the nucleus of the tractus solitarius (NTS) *in vivo*.

2. Three distinct patterns of postsynaptic responses were evoked by SN stimulation, an EPSP, an EPSP–IPSP sequence and an IPSP, observed separately in individual neurones. This diversity of response was represented in cells proven to receive baroreceptor input by inflation of a balloon-tipped catheter within the ipsilateral carotid sinus.

3. Virtually none of the neurones identified as baroreceptive exhibited pulse-related discharge.

4. A variety of influences to AN, SLN and VN stimulation were observed in neurones receiving baroreceptor afferent information. This wide convergence of input implies that this region of the brain stem is important in the integration of cardiovascular reflexes.

5. The hypothesis was tested that respiratory 'gating' of the baroreceptor reflex is produced by synaptic actions within the NTS. There was an absence of any modification of PSPs by lung inflation and by variations in the timing of the stimulation of the afferent nerves within the respiratory cycle. These observations indicate that respiratory modifications of the baroreceptor reflex must occur at later stages in the reflex pathway.

INTRODUCTION

There is a large body of both anatomical and physiological evidence that demonstrates the important role of the nucleus of the tractus solitarius (NTS) in the processing of cardiovascular and respiratory reflex inputs. Particular attention has

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been paid in determining the extent and localization of the baroreceptor afferent input to this nucleus (see Spyer, 1981 and Jordan & Spyer, 1986 for review). This appears to be directed to distinct regions of the nucleus extending rostrally from the obex and involving lateral and ventrolateral subnuclei as well as dorsomedial aspects of the nucleus (Donoghue, Garcia, Jordan & Spyer, 1982*a*; Donoghue, Felder, Jordan & Spyer, 1984). These regions also receive an innervation from slowly adapting vagal lung stretch afferent fibres (Donoghue, Garcia, Jordan & Spyer, 1982*b*) amongst other inputs (Jordan & Spyer, 1986). These observations have led to the belief that the integration of the reflex control of circulation and respiration is achieved through synaptic interactions at this level of the CNS (see Spyer, 1982) particularly as the dorsal group of medullary respiratory neurones are located within the ventrolateral subnucleus of the NTS (Merrill, 1974).

The consequences of these presumed interactions can be illustrated by considering the respiratory modifications of the baroreceptor reflex that have been demonstrated in numerous laboratories. A stimulus to the arterial baroreceptors will only excite cardiac vagal efferents if it is timed to occur during expiration; an equivalent stimulus arriving during inspiration is ineffective (e.g. Davidson, Goldner & McClosky, 1976). This is largely the result of a direct inspiratory control of the activity of vagal motoneurones (Gilbey, Jordan, Richter & Spyer, 1984) but the ability of lung stretch inputs to modify the performance of the reflex cannot be explained in that way (Potter, 1981) and was postulated to occur at an earlier site in the reflex pathway possibly within the NTS. In a recent study an attempt was made to determine whether a presynaptic action of vagal afferents could be observed on the terminals of baroreceptor afferents recorded in the NTS (Richter, Jordan, Ballantyne, Meesmann & Spyer, 1986). No evidence for such an action, which would have indicated a presynaptic 'gating' of the afferent input, was obtained and central respiratory activity was similarly ineffective (Richter *et al.* 1986). This indicates that the influence of lung stretch afferents is mediated at synapses between the primary afferent terminal and the vagal outflow. This has encouraged us to observe the influences of respiratory activity, both those arising from the central respiratory oscillator and reflex inputs resulting from respiratory movements, on the PSPs evoked in NTS neurones on activation on the sinus nerve (SN), and specifically the arterial baroreceptors. The present report will describe initially the range of postsynaptic actions that these inputs can exert on non-respiratory neurones of the NTS and represents the first detailed analysis using intracellular recordings that has yet appeared. There are, to our knowledge, only three other somewhat similar reports (Hildebrandt, 1974; Miura, 1975; Donoghue, Felder, Gilbey, Jordan & Spyer, 1985) but these did not specifically examine the actions of activating the carotid sinus baroreceptors. In the accompanying papers we examine the influence of stimulating within the hypothalamic defence area on the processing of the baroreceptor input within the NTS (Mifflin, Spyer & Withington-Wray, 1988). In the third report we describe the use of neuropharmacological techniques to determine the pharmacology of the inhibitory effect that is evoked from the hypothalamus (Jordan, Mifflin & Spyer, 1988). Together these papers provide a detailed analysis of the neural circuitry within the NTS that integrates the baroreceptor reflex.

Preliminary reports of parts of these studies have been presented to the Physiological Society (Mifflin, Spyer & Withington-Wray, 1986*a*, *b*).

METHODS

Experiments were performed on sixty-one female cats (1·6–3·8 kg body weight). Anaesthesia was induced with an I.P. injection of sodium pentobarbitone (Sagatal, May & Baker Ltd, 35–40 mg/kg). A femoral vein was then cannulated for the administration of supplementary anaesthetic and drugs. In forty-five cats anaesthesia was maintained using I.V. injections of pentobarbitone (1–3 mg/kg as required), but in the other sixteen cats, anaesthesia was maintained using α-chloralose BDH (10 mg/kg as required). As the observations that were made subsequently in the two groups appeared identical, no distinctions are made in the presentation of data (see Jordan *et al.* 1988 for discussion).

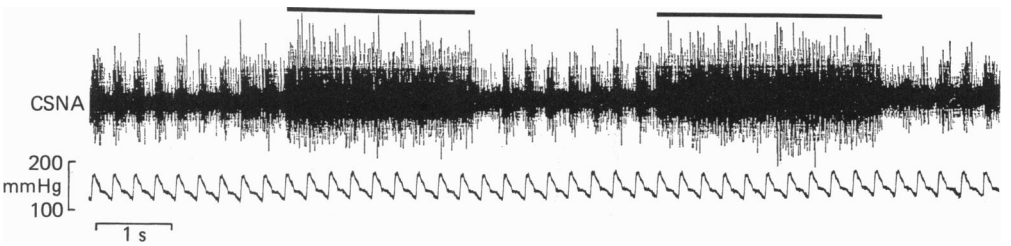

Fig. 1. Recording of carotid sinus nerve activity (CSNA) during inflation of the intra-sinus balloon (indicated by the bars over the neurogram). Femoral blood pressure displayed below nerve activity.

A femoral artery was cannulated for the measurement of arterial blood pressure. The trachea was intubated below the larynx and the animals were then ventilated artificially with O_2-enriched air (Harvard ventilator). The lungs were inflated to a maximal tracheal pressure of less than 10 mmHg, which was monitored through the side arm of the tracheal cannula, with an end-expiratory resistance of 1–2 mmHg to prevent collapse of the lungs during deflation. The rate of inflation was varied over a range of 15–25 per minute to maintain end-tidal CO_2 at approximately 4%, this being monitored from a second side arm of the tracheal cannula using an infra-red analyser (P. K. Morgan, 901 CO_2 Analyser). Blood gases were routinely monitored (Corning 158 pH/Blood gas Analyser) and deviations from normal physiological levels were corrected by varying ventilation and/or infusions of 1 M-sodium bicarbonate. Temperature was maintained at 38 ± 1 °C using a heating coil.

The right carotid sinus nerve (SN) was isolated using a lateral approach. The glossopharyngeal nerve was cut distal to its junction with the SN. An inflatable balloon-tipped catheter (Swan Ganz, American Hospital Supply Co.) was advanced via the external carotid artery into the region of the carotid sinus and positioned so that its inflation resulted in an increase in baroreceptor discharge (Fig. 1). Brief inflations (1–3 s) selectively activated the baroreceptors since: (1) There was no tonic discharge in the SN following balloon deflation and no change in phrenic nerve activity. This makes it unlikely that the carotid body chemoreceptors were activated. (2) During these periods of brief inflation there was no measurable change in arterial blood pressure minimizing the possibility that cardiovascular mechanoreceptors other than those of the carotid sinus were affected. That blood pressure was unaltered even during sustained (5–10 s) inflations makes it unlikely that the balloon inflation was maximally affecting all carotid baroreceptor endings. Failure to respond to balloon inflation is thus not a conclusive indication of either the absence of a baroreceptor, or presence of a chemoreceptor input to a particular neurone.

The cervical vagus (VN), the aortic nerve (AN) and the superior laryngeal nerve (SLN) on the right side were exposed and placed on bipolar silver stimulating electrodes. Nerves were stimulated with rectangular pulses of 0·1–1·0 ms duration delivered using a Digitimer (D4030) and isolated stimulators. For the SN and AN the intensity was set at 1·5–2·0 times the threshold for evoking a change in arterial pressure, whilst in the case of the SLN the cessation of phrenic activity was used to assess the efficacy of the stimulus. The right phrenic nerve having been isolated, cut and desheathed, the central end was placed on bipolar silver recording electrodes and covered with a mixture of paraffin and Vaseline.

The animal was placed in a stereotaxic head holder (Kopf) and suspended by thoracic and sacral vertebral clamps. The brain stem was exposed following an occipital craniotomy and gentle retraction of the cerebellum. To reduce respiratory-related movements of the brain stem a bilateral pneumothoracotomy was performed and the animal paralysed with gallamine triethiodide (Flaxedil, 4–8 mg/kg in an initial dose, 2–4 mg/kg per hour as a maintenance dose).

Extracellular and intracellular recordings were taken from neurones in the vicinity of the NTS. Penetrations were made in a region extending from 1 mm caudal to 3 mm rostral to the obex. The location of neurones was inferred from the identification of vagal motoneurones of the dorsal vagal nucleus and respiratory neurones of the ventrolateral subnucleus of the NTS. These were confirmed from the results of studies in which extracellular recording sites were marked by the deposition of Pontamine Sky Blue (Jordan, Mifflin & Spyer, 1987, 1988). Recordings were made from within a horse-shoe-shaped pressure foot (inner diameter 2 mm) placed on the medullary surface to enhance stability. Glass-filament microelectrodes were filled with either 3 M-potassium chloride or 2 M-potassium citrate and the tips broken to give DC impedances of 30–120 MΩ. In conjunction with another study (Mifflin, Spyer & Withington-Wray, 1987) some electrodes were filled with horseradish peroxidase (HRP, Sigma type VI) in Tris buffer containing 0·2 M-KCl (DC resistance 60–130 MΩ). Potentials were recorded using a DC bridge amplifier equipped with capacity compensation (Dagan 8100). Amplified DC recordings of membrane potential, arterial blood pressure, tracheal pressure, and a filtered AC record of phrenic nerve activity together with ECG were recorded on tape (Racal Store 7; frequency response DC to 5 kHz) and displayed on an electrostatic recorder (Gould ES 1000). The records presented in this paper are taken either from the latter recorder or represent data analysed off-line using a Digital oscilloscope (Nicolet Explorer 204a) and plotted on an X–Y plotter. Post-stimulus time histograms of extracellularly recorded spike trains and signal averages of membrane potential from intracellular recordings were constructed off-line using a spike processor (Digitimer D1300) and minicomputer (CED Slam System). Trigger signals were derived from the ECG on occasions. The statistical significance of difference was determined using a one-way analysis of variance.

RESULTS

The activity of several hundred neurones in the immediate vicinity of the NTS was recorded in this study. The great majority were analysed only with extracellular recordings but this paper will concentrate on the information obtained with intracellular recordings obtained in sixty-five neurones, many of which had also been studied prior to impalement. We chose to neglect neurones whose membrane potentials were less than −45 mV, as we observed that in cells with lower membrane potentials recordings were unstable, and there was a significant difference between the extracellularly recorded discharge (spontaneous and/or evoked) and that recorded intracellularly. The response of these neurones to electrical stimulation of the SN and a selection of other inputs (i.e. AN, SLN or VN) were studied and the effects of inflation of the balloon-tipped catheter in the ipsilateral carotid sinus were noted. Wherever possible this characterization was accomplished prior to impalement since the inflation of the balloon involved contact with the experimental table. For convenience the data are presented in relation to the pattern of postsynaptic events elicited by SN stimulation. Three distinct patterns of PSPs were evoked – an EPSP, an IPSP and an EPSP–IPSP sequence.

EPSPs in response to SN stimulation

In forty-three of the sixty-five neurones studied SN stimulation evoked an EPSP (membrane potentials = −47 to −64 mV). The latency to onset was 6·8 ± 4·3 ms (mean ± S.D.; n = 43, range 3·2–26·4 ms) and the amplitude was 3·9 ± 1·5 mV (range 1·9–8·6 mV) in the twenty-eight neurones in which it was possible to abolish action

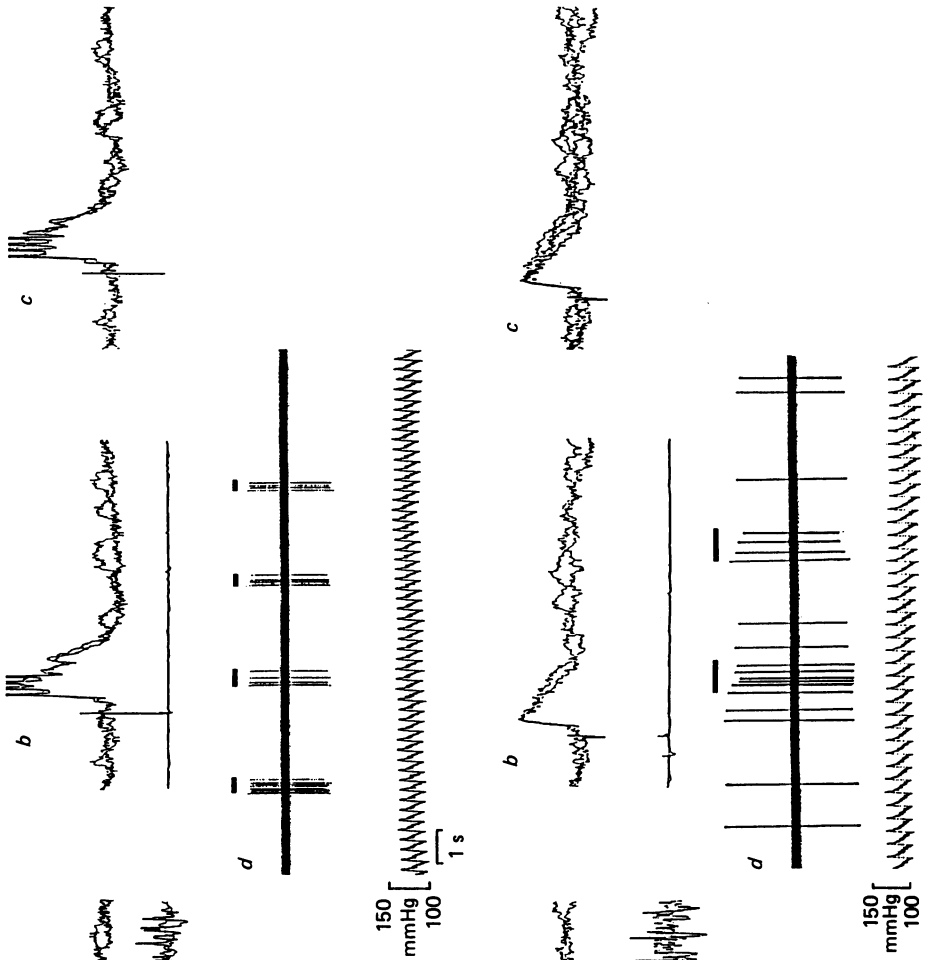

Fig. 2. EPSPs evoked by carotid sinus nerve stimulation (SN) in two cells excited by baroreceptor activation. The extracellularly recorded responses to inflation of the intra-sinus balloon (indicated by the bars over the unit recording) are illustrated in *A d* and *Bd*. *A a–c* and *Ba–c* illustrate the intracellularly recorded responses to SN stimulation in these neurones with phrenic nerve activity (PNA) displayed below. EPSPs evoked during inspiration (*A a* and *Ba*) and during expiration (*A b* and *Bb*), one sweep when the lungs were inflated superimposed with one sweep when the lungs were not inflated. *A c*, EPSP during inspiration (from *A a*) superimposed with one during expiration (from *A b*). *Bc*, sweeps in *Ba* superimposed with the sweeps in *Bb*. *A a–c*, action potentials discharged at the peak of the EPSP are truncated. Membrane potential: $A = -54$ mV, $B = -51$ mV. Potassium citrate-filled electrodes.

potential discharge (by hyperpolarizing DC current or reductions in stimulus intensity). In the case of these twenty-eight neurones the time to peak of the EPSP was $10{\cdot}2 \pm 8{\cdot}7$ ms. The latency and the rising phase of the EPSP were very consistent in each neurone although the duration of the EPSP was more variable (Figs 2 and 3). Increasing the intensity of stimulation decreased the latency to onset and increased the amplitude of the response and only occasionally altered the pattern of the response (see Fig. 5 and subsequent text). In seventeen out of the thirty-one neurones tested there was evidence of an excitatory input from the carotid sinus baroreceptors (Fig. 2). In these neurones, effective balloon inflations always evoked an excitatory response. The latency of the SN-evoked EPSP was shorter ($P = 0{\cdot}03$) in those neurones excited by balloon inflation ($5{\cdot}5 \pm 1{\cdot}8$ ms, range 3·2–8·9 ms, $n = 17$) as against $8{\cdot}1 \pm 4{\cdot}3$ ms (range 3·5–19·8 ms, $n = 14$) in those that were unaffected by balloon inflation. There was, however, no difference in the amplitude of the EPSPs of the two groups ($P = 0{\cdot}33$) being $4{\cdot}1 \pm 2{\cdot}0$ ms (range 1·9–8·2 mV) and $3{\cdot}3 \pm 0{\cdot}7$ mV (range 2·7–4·8 mV), respectively. Similarly the time to peak of the EPSPs was essentially the same in the two groups ($P = 0{\cdot}59$), the values being $9{\cdot}3 \pm 6{\cdot}3$ ms ($n = 10$) and $11{\cdot}4 \pm 9{\cdot}9$ ms ($n = 7$). In agreement with a previous report from this laboratory (Donoghue *et al.* 1985) a convergence of excitatory inputs from other peripheral afferents inputs was observed. This was not tested exhaustively but there appeared no difference in the incidence of convergence between those neurones receiving a baroreceptor input, and those that were unaffected by balloon inflation. Stimulation of the AN, SLN and VN were all effective (see Fig. 3) and in every case ($n = 12$) where SN stimulation evoked an EPSP so did the other input(s), although the stimulus intensity of the convergent inputs was not systematically varied.

When examined over a longer time frame, the membrane potential of these neurones showed no obvious rhythmic fluctuations entrained to the arterial pulse. Averages of membrane potential using the QRS complex of the ECG as a trigger were flat ($n = 11$) as were ECG-triggered histograms of extracellularly recorded ongoing discharge ($n = 18$). This was so in neurones that responded vigorously to balloon inflation (Figs 2 and 5) but is not inconsistent with other observations reported in the literature (Lipski, McAllen & Spyer, 1975). Pulse-related activity was observed in ten extracellularly recorded units and one intracellularly recorded cell (Fig. 4) and was convincingly shown not to be the consequence of movement artifacts. Of eight of these neurones tested only two responded to balloon inflation and only three of nine tested were seen to be excited by SN stimulation with a latency of 3·4–6·8 ms. This latency is within the range for non-pulse rhythmic neurones receiving SN-evoked EPSPs and excitatory inputs from the baroreceptors. In no case was pulse-

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Fig. 3. Action potentials (*A*) and EPSPs (*B*) evoked by carotid sinus nerve, vagal and superior laryngeal nerve stimulation in a cell depolarized during activation of the baroreceptors. In *B* membrane potential was hyperpolarized by 4 nA DC current and the stimulus intensities were slightly decreased from the levels in *A* so that the EPSPs were subthreshold for action potential generation. Two sweeps during inspiration (*A a* and *B a*) and during expiration (*A b* and *B b*), one when the lungs were inflated superimposed with one when the lungs were not inflated. Phrenic nerve activity (PNA) displayed below. *A c* and *B c*, two sweeps in column *a* superimposed with the two sweeps in column *b*. Resting membrane potential: −56 mV. HRP-filled electrode.

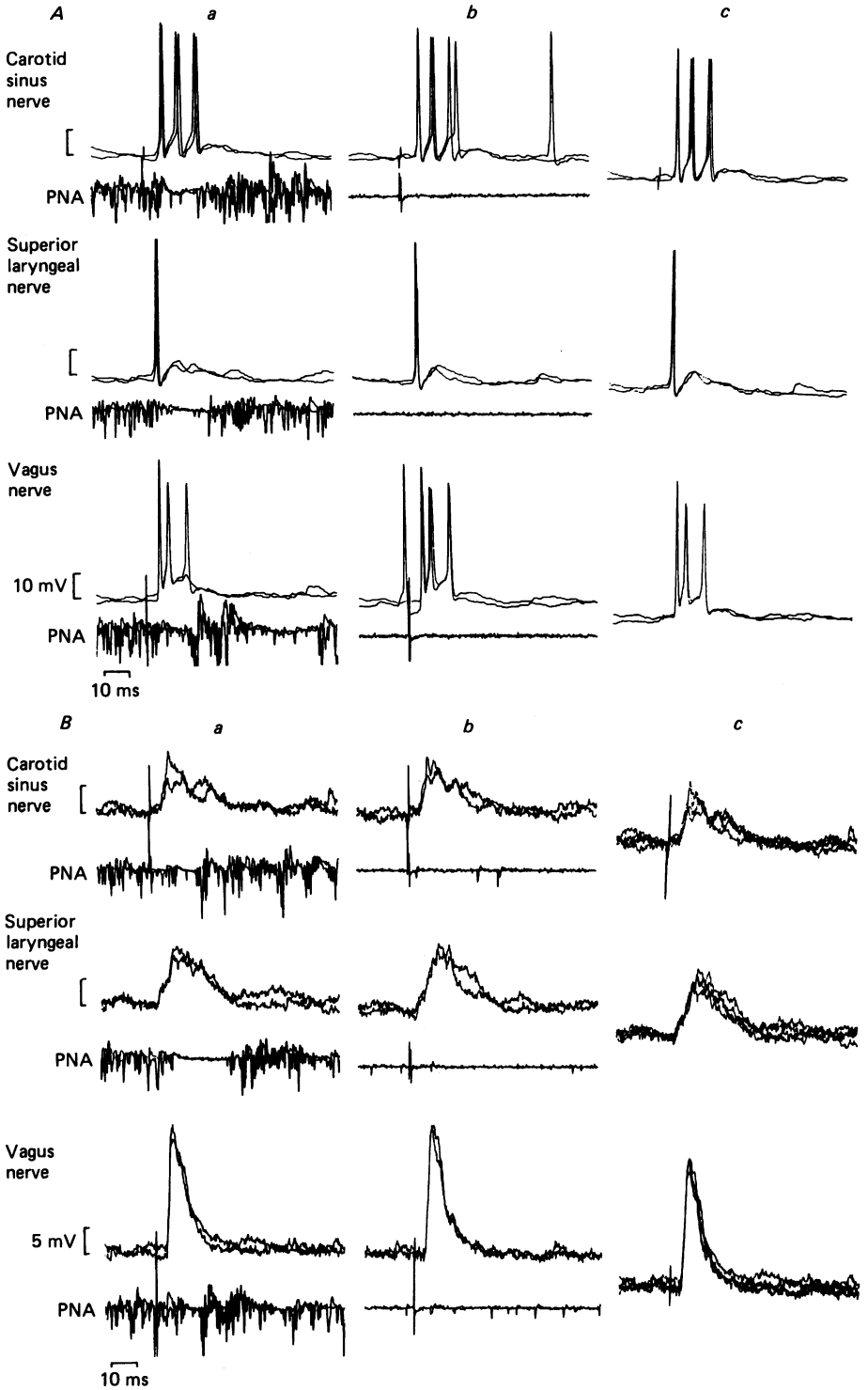

Figure 3. For legend see opposite.

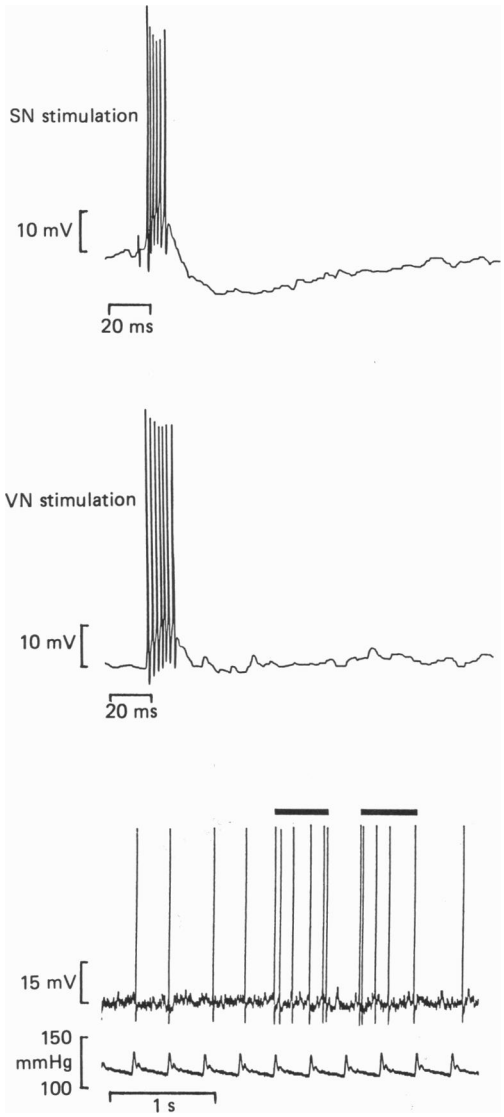

Fig. 4. EPSP–IPSP sequence evoked by carotid sinus nerve stimulation (SN; top trace) and EPSP evoked by vagal nerve stimulation (VN; middle trace) and a cell activated by baroreceptor stimulation (indicated by the bars; bottom trace). Membrane potential: −63 mV. Potassium citrate-filled electrode.

modulated activity seen extracellularly which was subsequently absent following impalement of the neurone.

EPSP–IPSP sequence in response to SN stimulation

Fourteen neurones exhibited an EPSP–IPSP sequence in response to SN stimulation (membrane potential = −49 to −66 mV). Three out of nine tested were excited and one inhibited by inflation of the intra-sinus balloon.

In six neurones it was not possible to eliminate action potential discharge evoked by SN stimulation, therefore the analysis of the membrane potentials has thus been restricted to the remaining eight neurones. The EPSP had on onset latency of $5{\cdot}2 \pm 1{\cdot}2$ ms (range 3·4–6·8 ms) and an amplitude of $3{\cdot}1 \pm 1{\cdot}3$ mV (range 1·4–5·1 mV). The amplitude of the subsequent IPSP was $3{\cdot}9 \pm 2{\cdot}4$ mV (range 1·6–9·2 mV). Several of these neurones also received convergent inputs from the AN, SLN or VN but the

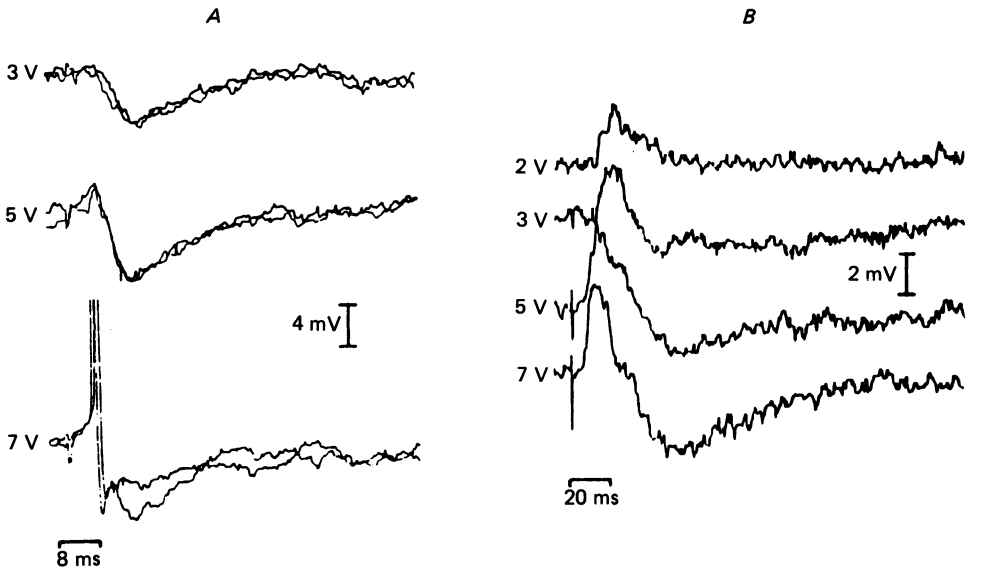

Fig. 5. Variation of carotid sinus nerve stimulation intensity in two cells in which carotid sinus nerve stimulation evoked an EPSP–IPSP sequence. Traces separated by an arbitrary DC level with the stimulus intensities, in volts (V) given to the left of each sweep. *A*, cell in which IPSP was lower threshold input. Two sweeps superimposed at each stimulus intensity. Action potentials at highest intensity truncated. Membrane potential: −55 mV. Potassium citrate-filled electrode. *B*, cell in which EPSP was lower threshold input. Membrane potential: −51 mV. Potassium citrate-filled electrode.

form of the convergent-evoked response did not necessarily conform to the EPSP–IPSP sequence observed in response to SN stimulation. Indeed in the case illustrated in Fig. 4 SN stimulation evoked a pronounced EPSP–IPSP sequence with superimposed spike discharge whilst VN stimulation elicited an EPSP of equivalent magnitude to that evoked from the SN, together with superimposed spikes, but no subsequent IPSP. This makes it unlikely that the secondary IPSP to SN stimulation is an activity-related phenomenon (Hotson & Prince, 1980). Supporting this conclusion is the fact that by varying the intensity of stimulation it was possible on occasions to generate IPSPs independently of the normally preceding EPSP (Fig. 5*A*) and they were often present in situations when SN stimulation failed to evoke discharge (Fig. 5*B*).

The intensity of the SN stimulus was varied to determine the thresholds of EPSP and IPSP components of the response (Fig. 5). In four out of nine neurones tested the EPSP had the lower threshold, with its latency decreasing and its amplitude increasing on raising the intensity and at a higher intensity the IPSP was evoked.

With further increases of intensity, the amplitude of both the EPSP and IPSP increased (Fig. 5*B*). At higher intensities the duration of the EPSP was reduced suggesting that a decrease in the onset latency of the enlarged IPSP was effectively shunting the later portion of the EPSP. In two of the nine neurones tested the IPSP had the lower threshold (Fig. 5*A*), the EPSP being recruited at higher stimulus intensities. In the remaining three neurones it was not possible to discriminate a difference in the thresholds of the two PSP components.

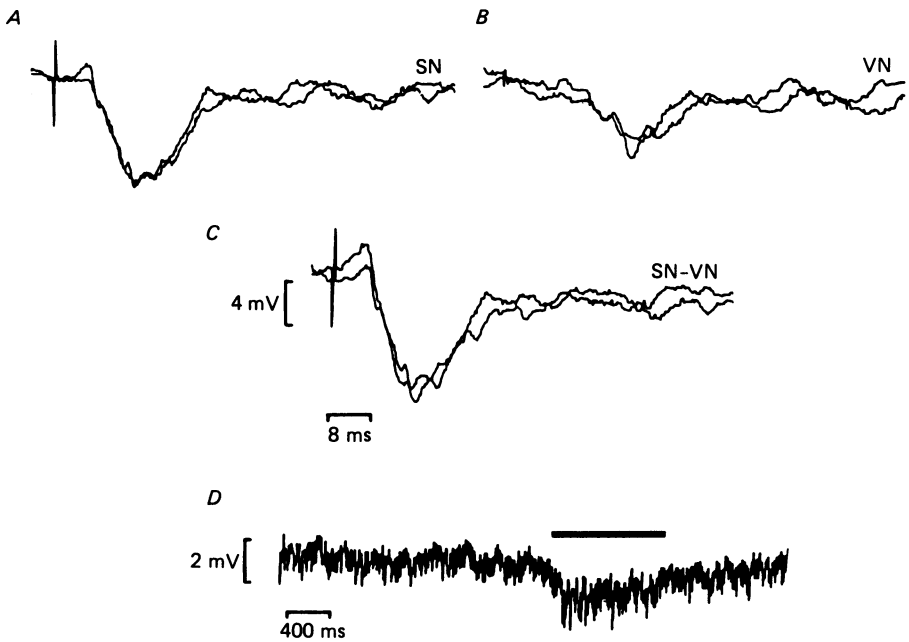

Fig. 6. *A*, EPSP–IPSP evoked by carotid sinus nerve stimulation (SN). *B*, IPSP evoked by vagal nerve stimulation (VN). *C*, response to simultaneous stimulation of SN and VN (SN–VN). Two sweeps superimposed in *A*, *B* and *C*. *D*, membrane potential response to baroreceptor activation during period indicated by the bar. Membrane potential: −59 mV. Potassium chloride-filled electrode.

The unit depicted in Fig. 6 was the only neurone that displayed an EPSP–IPSP sequence to SN stimulation and was inhibited on balloon inflation. The initial EPSP was present to only approximately 50% of stimuli delivered although the secondary IPSP was present to each stimulus (Fig. 6*A*). The neurone also received a long-latency IPSP from the VN (Fig. 6*B*) and when this stimulus was paired with SN stimulation the initial EPSP was present in every trial, although variable in amplitude, and the IPSP was increased in both amplitude and duration (Fig. 6*C*).

IPSPs in response to SN stimulation

With the exception of the neurone illustrated in Fig. 6, all neurones that were inhibited by balloon inflation, and hence received an inhibitory input from the baroreceptors, are included in this group. However, IPSPs that were evoked in these

neurones were indistinguishable in terms of latency, amplitude and duration from those evoked in neurones which were unaffected by balloon inflation. For the eight neurones (membrane potentials = −52 to −62 mV) shown to receive an exclusive IPSP on SN stimulation the latency was 9·4 ± 2·8 ms (range 6·3–15·0 ms) and the amplitude was 3·6 ± 1·2 mV (range 1·7–5·2 mV). In total three of six tested were shown to be inhibited by balloon inflation (see Figs 7 and 8*B*).

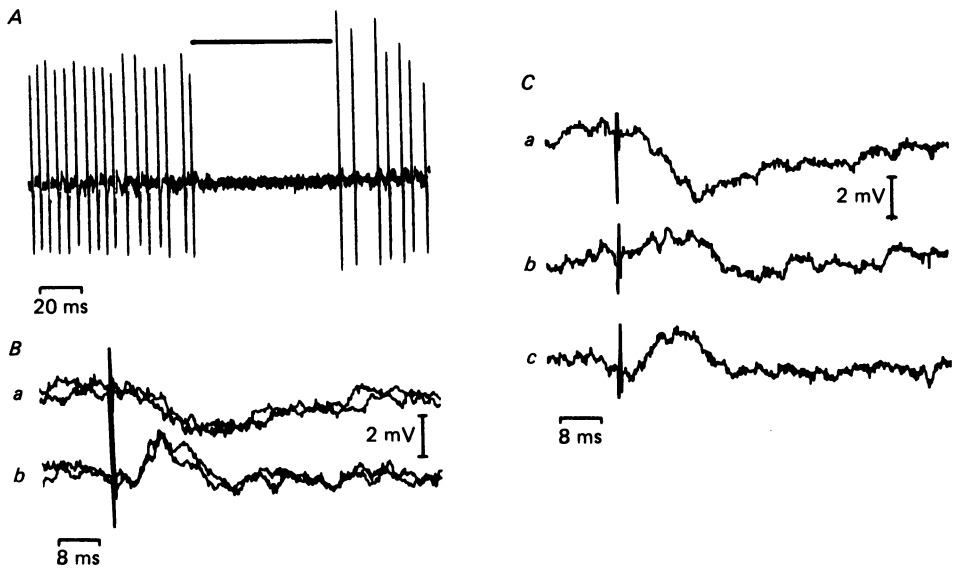

Fig. 7. Cell inhibited by baroreceptor activation and receiving IPSP following carotid sinus nerve (SN) stimulation. Potassium citrate-filled electrode. *A*, extracellularly recorded spontaneous activity abolished during baroreceptor activation (indicated by bar). *Ba*, IPSP evoked by SN stimulation. Membrane potential: −61 mV. *Bb*, IPSP reversed to depolarizing potential during passage of 6 nA hyperpolarizing DC current. Note the decreased duration compared to the control, hyperpolarizing IPSP in *Ba*. Two sweeps each superimposed in *Ba* and *Bb*. *Ca*, *b* and *c*: transition of control hyperpolarizing IPSP (*Ca*) to reversed depolarizing potential as hyperpolarizing current was gradually increased. In *Cb* note that the early portion of the IPSP reversed to a depolarizing potential before the later portion. The DC levels of the traces in *B* and *C* were shifted to aid visualization with the more hyperpolarized membrane potentials downwards.

The SN-evoked hyperpolarization was reversed to a depolarizing potential when hyperpolarizing DC current (3–10 nA) was applied to the neurone (Fig. 7*B* and *C*). The early portion of the hyperpolarizing potential reversed prior to the later component (Fig. 7*C*). Such a biphasic pattern of reversal is indicative of a distributed inhibitory synaptic input (Hubbard, Llinas & Quastel, 1969), an inference that is supported by the shorter duration of the reversed IPSP as compared with the control hyperpolarizing effect (Fig. 7*B*). In situations using KCl-filled electrodes the diffusion of Cl^- often resulted in the spontaneous reversal of hyperpolarizing potentials evoked from the SN (Fig. 8*A*) and the baroreceptors (Fig. 8*B*). These could be re-reversed with the application of depolarizing DC currents (Fig. 8*Ab*).

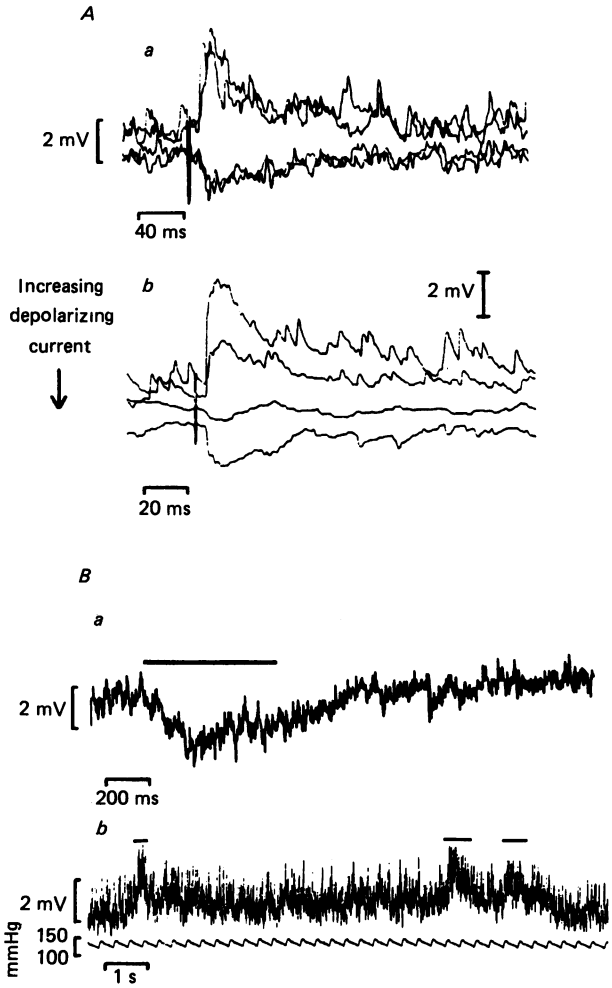

Fig. 8. Cell inhibited by baroreceptor activation and receiving IPSP following carotid sinus nerve stimulation (SN). Potassium chloride-filled electrode. *Aa*, two sweeps superimposed of initial hyperpolarization evoked by SN (membrane potential: −54 mV) and two sweeps of the spontaneously reversed IPSP. *Ab*, re-reversal of reversed depolarizing IPSP as depolarizing DC current was gradually increased. DC level of traces arbitrarily aligned with increasingly depolarized membrane potentials downwards. Note that the early phase of the IPSP re-reverses before the later phases and the spontaneous synaptic noise. *Ba*, initial hyperpolarization of membrane potential produced by baroreceptor activation (indicated by bar). *Bb*, after spontaneous reversal of SN-evoked IPSP, baroreceptor activation (indicated by bars) resulted in a depolarization of membrane potential. Arterial pressure is displayed below the membrane potential trace.

Under these conditions the early phase of the potential reversed before the later component and the ongoing synaptic noise. Note also the re-reversal of this synaptic noise, indicating that the cell received tonic, inhibitory inputs. In two cases neurones were seen to receive convergent inhibitory inputs from two or more inputs. This is at variance with data presented in a recent publication (Donoghue *et al.* 1985).

The influence of respiration on CSN-evoked PSPs

To determine if SN, and specifically baroreceptor, inputs to the NTS were modified during the respiratory cycle the PSPs evoked by SN stimulation were examined during both the inspiratory and expiratory phases of central respiratory activity and during periods of lung inflation and deflation. Since the animals were paralysed and artificially ventilated, the lungs were inflated independently of central respiratory activity. This allowed the examination of the effects of periods of lung inflation during both inspiratory and expiratory phases of the central respiratory cycle. SN stimulation produced an inhibition of phrenic nerve discharge (latency 5–15 ms, duration 10–30 ms; see Figs 2, 3 and 9) as described previously (Biscoe & Sampson, 1970). However, in no instance was an SN-evoked PSP – be it EPSP, EPSP–IPSP or IPSP – altered in any way by central respiratory activity or the state of inflation of the lung. The latency and time to peak of the PSPs remained unaltered (Figs 2, 3 and 9) and both subthreshold and suprathreshold effects were similarly uneffected (Fig. 3*A* and *B*), as were convergent inputs from other peripheral reflexogenic areas (Fig. 3). This lack of effect was observed in all cells in which PSPs were analysed throughout the respiratory cycle. This involved thirty-two cells in which SN stimulation evoked an EPSP (Figs 2 and 3) of which fourteen out of twenty-nine tested were excited by balloon inflation. Ten cells which received an EPSP–IPSP sequence to CSN stimulation were also unaffected and this was so in those neurones receiving an exclusive IPSP ($n = 6$) of which two out of five tested showed a baroreceptor-evoked IPSP (Fig. 9*B*). Observing the membrane potential of all these neurones on a slow time scale failed to reveal any subtle alterations in either membrane potential or synaptic noise at any particular phase of the respiratory cycle. Lung inflation was similarly ineffective in altering these variables.

DISCUSSION

The data presented in this report represent the first detailed description *in vivo* of the postsynaptic action of SN, and other cardiovascular and respiratory afferent inputs, on NTS non-respiratory neurones. The three distinct patterns of postsynaptic response – EPSP, and EPSP–IPSP sequence and IPSP – observed separately in individual neurones appear to reflect the patterns generated by activation of the carotid sinus baroreceptors also. The clear evidence of convergence of afferent input onto these neurones from several different sources indicates that we are dealing with an important integrative neural network that is of significance in cardiovascular control. This is further emphasized by the results of a complementary study that details the effects of activating the hypothalamic defence area on the activity of NTS neurones and specifically its influence on the effectiveness of the inputs described here (Mifflin *et al.* 1988).

The latencies and amplitudes of the evoked PSPs are within the range to be expected on the basis of both previous *in vivo* studies (Donoghue *et al.* 1985) and from observations in *in vitro* preparations (Miles, 1986; Champagnat, Denavit-Saubie, Grant & Shen, 1986). In the *in vitro* studies postsynaptic responses in NTS neurones were generated by activating fibres within the tractus solitarius, which will not

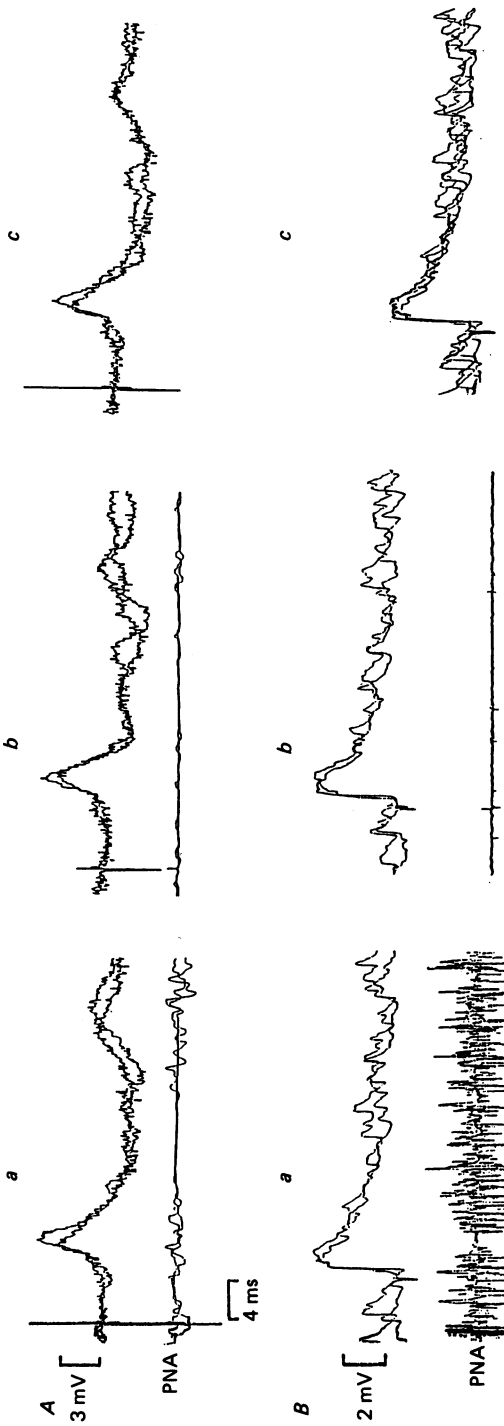

Fig. 9. EPSP–IPSP (*A*) and reversed IPSP (*B*) (from same cell as in Fig. 8) evoked by carotid sinus nerve stimulation during the respiratory cycle. Phrenic nerve activity (PNA) is displayed below the PSPs in panels *a* and *b*. Two PSPs during inspiration (*A a* and *B a*) and during expiration (*A b* and *B b*), one when the lungs were inflated and one when the lungs were not inflated. *A c*, one PSP during inspiration (from *A a*) superimposed with one PSP during expiration (from *A b*). *B c*, the two PSPs in *B a* superimposed with the two PSPs in *B b*. Membrane potential: $A = -53$ mV, $B = -52$ mV.

provide a means of establishing a functional distinction of the afferents involved in generating the responses.

With regard to the exclusive EPSP response there are similarities in the characteristics of the PSPs observed in the present study and those generated in neurones defined as the 'post-spike increased excitability neurone' (Champagnat *et al.* 1986) although we saw no evidence of prolonged secondary waves of depolarization that would account for heightened excitability. The description of 'post-spike reduced excitability neurones' in Champagnat *et al.* (1986) is certainly consistent with our observations of neurones receiving an EPSP–IPSP sequence. There is, however, no equivalent in that, or other *in vitro* reports, for the exclusive IPSP response that we have described. This may reflect either the different sources of afferent input studied in the two different situations (i.e. *in vivo* as distinct from *in vitro*) or simply that the different preparations bias one towards revealing inhibitory processes (the *in vivo* situation) to one in which inhibition is less effectively expressed (the *in vitro* preparation). In the report of Miles (1986) it was stressed that IPSPs although generated with characteristics of disynaptic actions invariably followed an initial EPSP. This is also at variance with our observations of EPSP–IPSP sequences where it was often the case that the secondary IPSP had a lower threshold than the preceding EPSP.

For the IPSPs recorded, both those evoked without an accompanying EPSP and those following an EPSP, we have evidence that they are mediated by a Cl^--dependent process since the injection of hyperpolarizing currents and Cl^- led to a reversal of the hyperpolarizing to a depolarization. The biphasic reversal of IPSP polarity as hyperpolarizing current intensity was gradually increased is suggestive evidence for a widely distributed inhibitory synaptic input to the cell (Hubbard *et al.* 1969).

EPSP and IPSPs have been reported on stimulating within the tractus solitarius in the *in vitro* preparation (Miles, 1986). Then the resting membrane potential of the recorded cells were so close to the equilibrium potential of the IPSP that little or no hyperpolarization was noted and the inhibitory action was postulated to result as a consequence of the induced conductance changes. In the *in vivo* situation we observed significant changes in membrane potential.

Considering the EPSPs generated by both SN and baroreceptor stimulation it is perhaps surprising that virtually none of the neurones so affected gave any indication of having a pulse-related discharge or rhythmic fluctuation in membrane potential. Previous reports from this laboratory have also commented on the paucity of neurones demonstrating such rhythms and also having SN and baroreceptor inputs (see review by Jordan & Spyer, 1986), a finding reported in numerous other studies (see for example Hildebrandt, 1974; Miura, 1975 amongst others fully cited in Spyer, 1981). The marked level of ongoing, spontaneous synaptic activity which we have observed in these *in vivo* recordings suggests that a tonic level of synaptic depolarization is maintained by subthreshold excitatory inputs. There is also evidence for tonic GABAergic inhibitory inputs (see Jordan *et al.* 1988). This might explain the non-pulse rhythmic discharge given the varying conduction velocities of the fibres mediating the afferent input from the baroreceptors, the interactions beween them and other inputs in those cells showing convergent inputs, tonic

descending inhibitory inputs and the low-pass characteristics of the presumed first synapse in the reflex (Seller & Illert, 1969; Miles, 1986). Where pulse-modulated discharge has been reported in the literature (Langhorst, Stroh-Werz, Dittmar & Camerer, 1975; Czachurski, Lackner, Ockert & Seller, 1982), no evidence has been provided to show that the neurones were activated by stimulation of the SN, AN or arterial baroreceptors. In some more recent, and as yet unreported studies, neurones with pulse-modulated activity, SN and baroreceptor inputs have been localized to a region of the NTS just dorsal to the tractus solitarius at a level 1–1·5 mm rostral to the obex (M. P. Gilbey, P. Izzo & K. M. Spyer, unpublished observations). This could imply that the responses evoked in the present report were mediated via a group of interneurones and that they were polysynaptically generated. Most certainly the pattern of EPSP in terms of latency, rise time and duration is consistent with a monsynaptic input to at least some of these cells, as the latency was less than 4 ms (see Lipski *et al.* 1975), from the arterial baroreceptors. An alternative interpretation is that we are viewing a widely distributed monosynaptic input involving the soma and both distal and proximal dendrites.

A major purpose of this investigation was to test the hypothesis that the well-established respiratory 'gating' of the baroreceptor reflex is produced by synaptic actions within the NTS. Previous studies appeared to have eliminated the possibility that the baroreceptor afferents themselves (either aortic or carotid) were under the presynaptic control of respiratory inputs (Rudomin, 1967; Barillot, 1970; Jordan & Spyer, 1979; Richter *et al.* 1986). These data indicate that the population of NTS neurones studied here are unaffected also. We could find no evidence that PSPs generated in NTS neurones by stimulating the SN, AN and VN were modified by lung inflation or the timing of the stimuli applied to these nerves with respect to the central respiratory cycle. This applied to those neurones that were shown to receive a baroreceptor input and also those which were unaffected by such stimulation, some proportion of which might receive a chemoreceptor input. The absence of any effect of central respiratory inputs was not unexpected in the light of the observations of Gilbey *et al.* (1984) that vagal cardioinhibitory neurones are directly inhibited in phase with inspiration. This ensures that the excitability of these neurones is set by respiratory state and this can adequately explain the respiratory modifications of the baroreceptor–vagal reflex. In view of Potter's observations in the dog (Potter, 1981) that lung stretch inputs could modify the baroreceptor control of the vagal outflow whilst failing to alter the tonic discharge of vagal cardiomotor neurones, it is surprising that no effect of lung inflation could be demonstrated especially as in a number of studies in different species a marked effect on baroreceptor regulation of heart rate and other cardiovascular variables has been reported (Daly, 1985 for references). However, in a recent study Daly, Ward & Wood (1986) have shown that the effects of loading and unloading the baroreceptors on the vascular resistance of the isolated and perfused hindlimb were unaffected by changing the level of pulmonary inflation. Taken together with Potter's observations on the influence of lung inflation on baroreceptor inputs to cardiac vagal motoneurones, it is likely that the action of lung inflation inputs is at a point after the decussation of the reflex into vagal and sympathetic limbs. This may be in the vicinity of the NTS or beyond but our current observations would seem to be limited to neurones closer to the afferent input.

Any action of lung stretch inputs evoking the type of 'gating' implicit in Potter's model would suggest that NTS neurones excited by SN, and baroreceptor, inputs would receive a convergent IPSP on VN stimulation and would exhibit a hyperpolarization in phase with lung inflation. No neurone receiving an EPSP on CSN stimulation received an IPSP on VN stimulation. The cell depicted in Fig. 6 with an EPSP–IPSP sequence on SN stimulation did receive an IPSP on VN stimulation but this was exceptional and the cell was *inhibited* by specific baroreceptor stimulation. The failure to reveal this sort of interaction reproducibly could result from a distally arranged synaptic input from vagal afferents but then it would be expected that whilst the amplitude and onset latency of the SN-evoked response would be unaltered the duration of the excitatory response would be markedly affected (Rall, Burke, Smith, Nelson & Frank, 1967; Jack, Noble & Tsien, 1975; Sypert, Munson & Fleshman, 1980). There was, however, no indication of any such change in the form of the PSP. The timing of the various synaptic inputs is critical when attempting to reveal this sort of interaction. In this respect we did not establish a detailed condition–test paradigm but feel that since we observed, and averaged, responses from randomly timed stimuli occurring at different points in both cycles, and made measurements over many such cycles, any physiologically significant changes would have been revealed. We must then conclude that any action of lung stretch inputs on the transmission of the baroreceptor, and chemoreceptor, reflex must be directed to other neurones in the NTS, or elsewhere in the brain stem, which are involved in the central processing of these afferent inputs.

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