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Hypothalamic modulation of the arterial chemoreceptor reflex in the anaesthetized cat: role of the nucleus tractus solitarii

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1. There is evidence in the literature of a mutual facilitatory interaction between the arterial chemoreceptor reflex and the alerting stage of the defence reaction, particularly in relation to the patterning of cardiorespiratory activity. The present study has been designed to test the hypothesis that a portion of this interaction involves synaptic interactions within the nucleus tractus solitarii (NTS).

2. The study has involved an analysis of the effective interactions between the stimulation of the arterial chemoreceptors and the hypothalamic defence area (HDA) on the activity of NTS neurones recorded in anaesthetized, paralysed and artificially ventilated cats.

3. A group of eighteen NTS neurones was classified as chemosensitive, on the basis of displaying EPSPs on sinus nerve stimulation (SN) and their failure to show an excitatory response to baroreceptor stimulation. Thirteen of these neurones displayed pronounced excitatory responses to chemoreceptor stimulation. In sixteen of these neurones HDA stimulation elicited an EPSP; in four of these sixteen neurones this early EPSP was followed by an IPSP. In the remaining two (of 18) neurones HDA stimulation provoked no obvious synaptic response but facilitated the efficacy of both chemoreceptor inputs and SN stimulation.

4. Neurones shown to receive convergent inputs from the arterial chemoreceptors (and SN stimulation) and HDA, often displayed excitatory responses to stimulation of other peripheral inputs. Vagally evoked EPSPs were observed in nine neurones, SLN-evoked responses in seven neurones and aortic nerve-evoked EPSPs in three neurones.

5. The organization of these synaptic interactions is discussed and these data are used to explain the pattern of interaction between chemoreceptor, baroreceptor and HDA inputs within the NTS. Conclusions are drawn regarding the functional role of different classes of NTS neurone, based on the findings in this and the accompanying two papers.

There is a small but compelling literature describing the interplay between the arterial chemoreceptor reflex and the defence response (see Marshall, 1987 for review). Indeed, it has long been recognized that activating the arterial chemoreceptors can lead to bursts of sham rages in the high-decerebrate animal (Bizzi, Libretti, Malliani & Zanchetti, 1961) and that the cardiovascular features of the chemoreceptor reflex resemble those associated with the alerting stage of the defence response (Marshall, 1987). This has led to the suggestion that there is a mutually facilitatory interaction between these two mechanisms and that this involves interactions within the CNS. This was demonstrated most clearly by Hilton & Joels (1965), who showed that stimulation in the hypothalamic defence area (HDA) of the cat led to a facilitation of the cardiorespiratory effects of activating the chemoreceptors of the carotid body. More recently, a brief report indicated that at least a portion of this effect can be attributed to interactions within the nucleus tractus solitarii (NTS) that were shown to involve a facilitation of sinus nerve-evoked responses when these were preceded by conditioning stimuli applied to the HDA (Silva-Carvalho, Dawid-Milner, Goldsmith & Spyer, 1993). The neuronal interconnections within the NTS that may account for such interactions have been reviewed in an accompanying report (Silva-Carvalho, Dawid-Milner & Spyer, 1995).

Chemoreceptor afferents project to restricted sites in the NTS (Donoghue, Felder, Jordan & Spyer, 1984) and make synaptic contact with neurones, particularly in the medial

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and commissural subnuclei, although chemosensitive neurones are distributed much more widely through the NTS (Spyer, Izzo, Liu, Paton, Silva-Carvalho & Richter, 1990; Mifflin, 1992, 1993). There is a degree of convergence of chemoreceptor and baroreceptor inputs onto NTS neurones (Lipski, McAllen & Trzebski, 1976; Mifflin, 1993) that may extend to as many as 44% of neurones receiving an SN input. Chemoreceptor-activated neurones are inhibited by baroreceptor inputs (Mifflin, 1993; Felder & Mifflin, 1994).

As mentioned already, the effects of chemoreceptor activation can be facilitated by a conditioning stimulus applied to the HDA (Silva-Carvalho *et al.* 1993), a stimulus that also inhibits the baroreceptor reflex (Mifflin, Spyer & Withington-Wray, 1988; Silva-Carvalho *et al.* 1995). The underlying synaptic mechanisms responsible for this reciprocal control of baroreceptor and chemoreceptor function have not as yet been analysed electrophysiologically. The current experiments have thus sought to identify, with intracellular recordings, the effects of hypothalamic stimulation on a selected population of NTS neurones that are activated by both sinus nerve stimulation and natural stimulation of the arterial chemoreceptors. These effects will then be compared with those that have been established for the hypothalamic control of baroreceptor-sensitive neurones in the NTS (Mifflin *et al.* 1988; Jordan, Mifflin & Spyer, 1988; Silva-Carvalho *et al.* 1995).

METHODS

These studies were undertaken on nine of the cats described by Silva-Carvalho *et al.* (1995).

In brief, cats were anaesthetized with sodium pentobarbitone (Sagatal; 40 mg kg^{-1}, initial dose I.P., supplemented when necessary with 4–5 mg doses I.V.). Balloon-tipped catheters were placed in the descending aorta via a femoral artery and the carotid sinus via the external carotid artery. In the former case, inflation increased vascular resistance so as to provoke a baroresponse, whilst in the latter inflation provoked a selective activation of the baroreceptors of the carotid sinus. Conversely, the arterial chemoreceptors of the carotid body were activated by injection of either small doses of 0·1% sodium cyanide or CO_2-saturated saline via the central lumen of the catheter positioned in the external carotid artery. The vagus (VN), aortic (AN) and sinus (SN) nerves were identified and isolated for electrical stimulation (1–2 pulses, 0·1 ms, 1–20 V given at 1 Hz) and the hypothalamic defence area was stimulated using a concentric bipolar electrode.

Animals were paralysed with gallamine triethiodide (Flaxedil; 4 mg kg^{-1} I.V., supplemented with 1–3 mg kg^{-1} h^{-1} I.V.). The level of anaesthesia was assessed by observing the presence or absence of a significant withdrawal reflex to pinching a paw before paralysis and by the absence of alterations in blood pressure, phrenic nerve activity and heart rate. Throughout the experiment, a stable level of these variables was used as an indication of the anaesthetic level and any change under resting conditions was countered by supplemental anaesthetic doses as described above. All procedures are described in full by Silva-Carvalho *et al.* (1995) and Dawid-Milner, Silva-Carvalho, Goldsmith & Spyer (1995).

For statistical comparison of respiratory and heart rate responses to stimulation of carotid chemoreceptors, with and without simultaneous stimulation of the HDA, Student's t test for paired observations was used. Values of t corresponding to $P < 0·05$ were considered to be significant. This test was also used to compare the responses to SN stimulation (latencies) before and after HDA stimulation.

RESULTS

Cardiorespiratory interactions

Previous reports have indicated that there is a mutual facilitatory interaction between the arterial chemoreceptor reflex and the response to HDA stimulation. In the present experiments the intracarotid injection of sodium cyanide or CO_2-saturated saline (volume, 0·1 ml) provoked an increase in phrenic nerve activity. This was accompanied by a rise in both arterial blood pressure and heart rate (see Fig. 1*A*). HDA stimulation (1 ms, 100 μA, 100 Hz, 5 s) elicited a similar pattern of response (Fig. 1*B*) and concomitant stimulation of the chemoreceptors and the HDA led to an intensified and more prolonged response (Fig. 1*C*). In six tests performed in different cats the duration of the first burst of phrenic nerve activity, observed after chemical excitation of the carotid body, was significantly increased by simultaneous stimulation of the HDA (from 4·21 ± 0·042 to 7·47 ± 0·48 s; $P < 0·001$). Both values were also significantly different from the duration of the burst of phrenic activity observed during HDA stimulation (5·1 ± 0·05 s). Furthermore, the increase in heart rate evoked by simultaneous activation of the HDA and carotid body (21·5 ± 2·43 beats min^{-1}) was significantly larger ($P < 0·001$) than the sum of individual effects of the separate stimuli (14 ± 2·43 beats min^{-1}). These observations strongly suggest a facilitatory interaction of these two inputs within the CNS.

Intracellular recordings in the NTS

This study was undertaken on a selected population of NTS neurones (membrane potential, −45 to −80 mV; $n = 18$ neurones) that were not excited by balloon inflation in either the carotid sinus or the descending aorta (Fig. 2*A*). These neurones showed no evidence of respiratory rhythm in their discharge (or membrane potential), nor were they modulated by lung inflation. These neurones were all excited on sinus nerve (SN) stimulation (latency, 5–15 ms; mean, 7 ms; and threshold to SN stimulation, 1–6 V). In the thirteen neurones of this group that were tested fully, chemoreceptor stimulation elicited an increase in discharge (Figs 2*A* and 4*D*) or membrane depolarization (Fig. 3*D*). These neurones, and an additional five that were classified as chemosensitive solely on the basis of their excitatory response to SN stimulation and the absence of an

excitatory input from the arterial baroreceptors, were then tested for their responses to HDA stimulation. In four of the eighteen neurones the SN-evoked EPSP was followed by an IPSP and in three of these baroreceptor stimulation led to a hyperpolarization.

In twelve of these neurones HDA stimulation evoked EPSPs and associated action potentials (Fig. 2*C*, *D* and *E*). The latency of the onset of the excitatory response was 4–16 ms (mean, 9 ms). On two occasions both long- (40 ms) and short-latency (less than 18 ms) responses to HDA

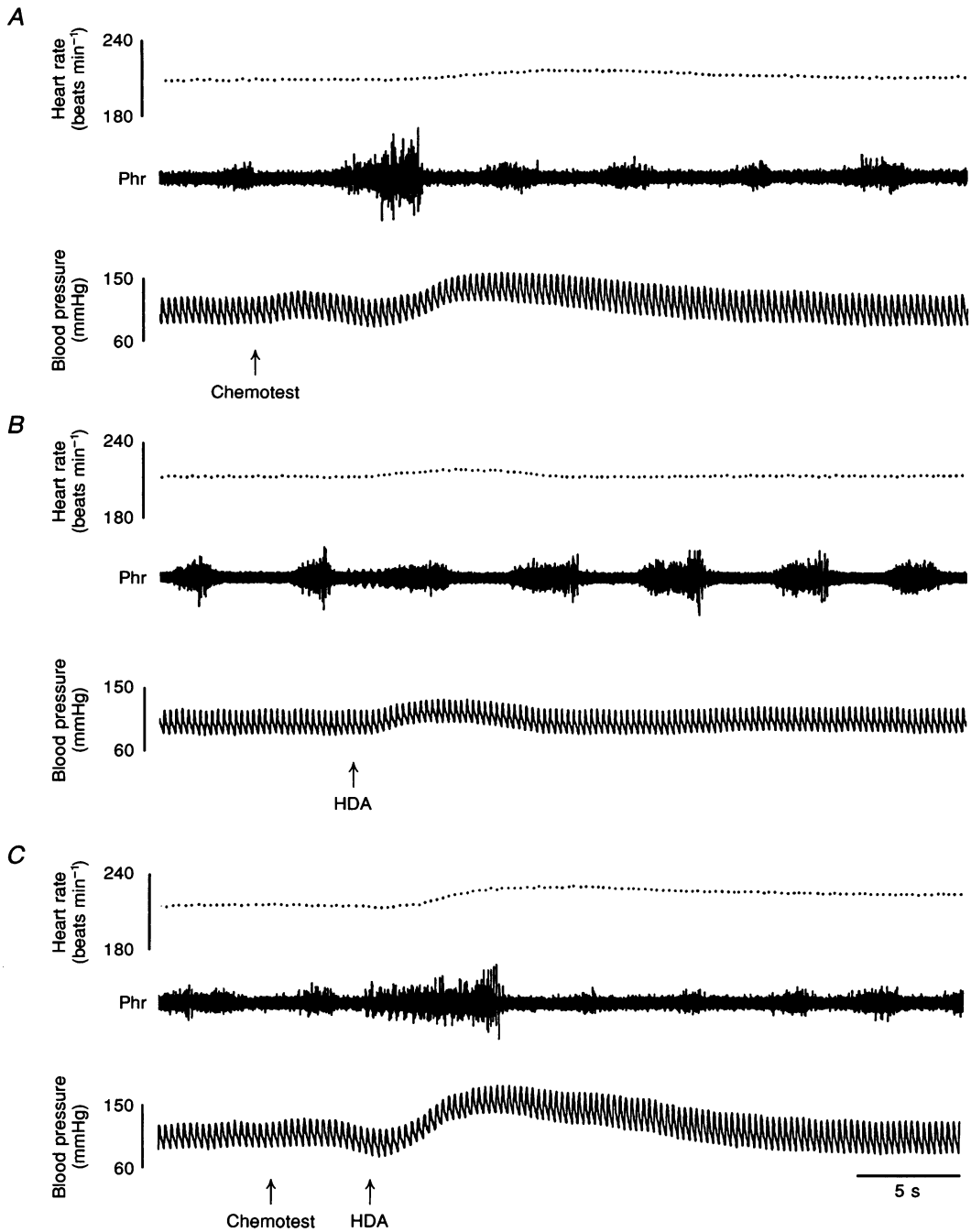

Figure 1. Interaction between chemoreceptor and HDA stimulii

A, the effect of an intracarotid injection of 0·1 ml of 0·1 % sodium cyanide (Chemotest). *B*, the effect of stimulation in the hypothalamic defence area (HDA; 100 μA at 100 Hz, 5 s) eliciting a submaximal response. *C*, simultaneous stimulation of the hypothalamus and the carotid body chemoreceptors (parameters as in *A* and *B*). Phr, phrenic nerve activity.

stimulation were observed in a single neurone (Fig. 4*A*). Four neurones exhibited an EPSP–IPSP on HDA stimulation (latency of the EPSP, 5, 5, 6 and 8 ms; latency of the IPSP, 20, 22, 30 and 40 ms, respectively), but in two neurones no HDA-evoked synaptic responses were observed even on increasing the intensity and/or duration of stimulation. In these latter cases, however, the HDA stimulus facilitated the effects of SN stimulation. In one case the EPSP evoked by SN stimulation was increased from 4 to 10 mV by the conditioning stimulus of HDA. In the other case (Fig. 3) HDA stimulation provoked a decrease in the latency of the onset of the EPSP evoked by SN stimulation (from 10·5 to 8 ms) as well as an increase in the duration of the EPSP (from 8·5 to 11·5 ms). In this case, SN stimulation had elicited an EPSP–IPSP (see Fig. 3). The neurone displayed low on-going discharge and was rarely brought to threshold by SN stimulation. Chemoreceptor stimulation evoked a membrane depolarization (maximum, 7 mV; Fig. 3*D*) which was long lasting, whilst aortic balloon inflation, which elevated arterial pressure to the same level as that evoked by chemoreceptor stimulation, elicited a membrane hyperpolarization (maximum, 5 mV) indicating an inhibitory input from arterial baroreceptors (Fig. 3*C*). These eighteen neurones were also tested for their responses to other inputs, evoked excitation being observed in response to VN stimulation in three neurones.

The pattern of response of those NTS neurones shown to receive convergent excitatory inputs from both SN and HDA (Figs 2 and 4) are clearly consistent with the facilitatory interactions between these two inputs (Fig. 1). Facilitatory responses are represented by a decrease in the latency of the onset of the response to SN stimulation provoked by the conditioning HDA stimulus (from

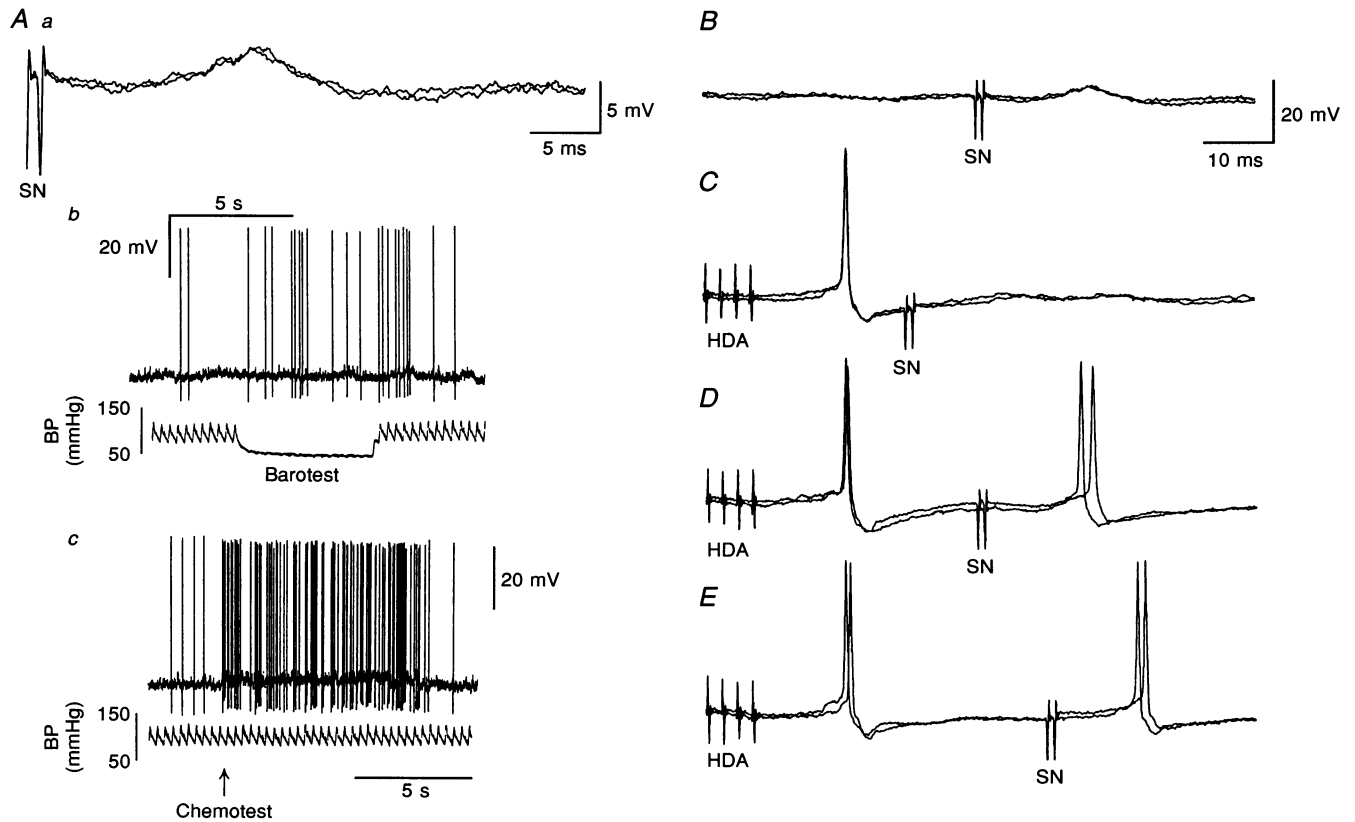

Figure 2. Responses of NTS neurone to HDA and SN stimuli

Intracellular recording taken from an NTS neurone (membrane potential, −45 mV). *A a* and *B*, response to sinus nerve stimulation (SN; 2 pulses, 0·1 ms, 1 kHz, 1 ms, 9 V given at 1 Hz). Note different recording speeds. Two superimposed traces. *A b* and *A c* show the responses of this neurone to aortic balloon inflation (Barotest; *A b*) (Note that balloon inflation occluded the cannula so that the blood pressure (BP) record does not represent the actual blood pressure); and to arterial chemoreceptor stimulation (Chemotest; *A c*). In *A b* and *A c* the upper trace shows the neuronal discharge and the lower trace shows the arterial blood pressure. *C*, *D* and *E*, stimulation of SN (as in *A* and *B*) 20, 30 and 40 ms after a conditioning stimulus to the HDA (4 pulses, 0·1 ms, 500 Hz, 100 μA given at 1 Hz). Note in *C* the suppression of the response during the after-hyperpolarization, and facilitation in *D* and *E*.

7·2 ± 2·5 to 5·3 ± 2·3 ms, $P < 0·001$). In four units in which SN stimulation evoked only an EPSP (mean, 5 mV), the combined conditioning stimulus evoked an action potential. In six cases stimulation of the HDA increased the number of action potentials evoked by SN stimulation (from 2·8 ± 1·7 to 4·8 ± 2·1, $P < 0·05$). In Fig. 2 evidence of an enhancement of the SN-evoked response is provided (Fig. 2*D* and *E*) at quite long conditioning–test intervals (circa 30–40 ms), although the after-hyperpolarization often obliterated SN excitatory influences (Fig. 2*C*). In Fig. 4 the effects of the HDA conditioning stimulus can be seen to provoke a markedly enhanced response to SN stimulation (compare Fig. 4*B* and *C*). The timing of arrival of these inputs affected the pattern of convergence and in the neurones illustrated in Figs 2 and 4 and others, potent effects could be seen with short conditioning–test intervals or simultaneous stimulation (not illustrated) as well as at the longer intervals shown.

The effects of the timing of conditioning–test intervals on the pattern of convergence were even more significant in the four neurones in which HDA stimulation evoked a complex response comprising an EPSP–IPSP sequence. The pattern of convergence could thus be either facilitatory if EPSPs from SN and HDA were timed to coincide, or inhibitory if the SN-evoked EPSPs were timed to coincide with HDA-evoked IPSPs (see Silva-Carvalho *et al.* 1995). However, in all eighteen neurones evidence of enhancement

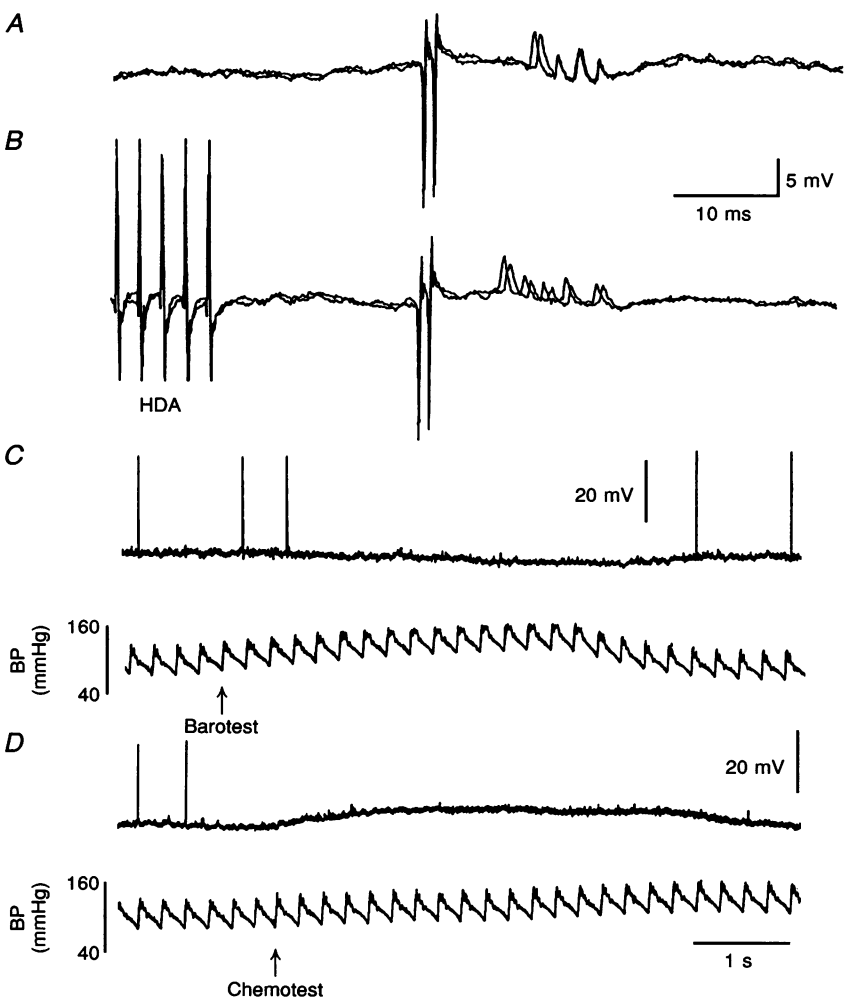

Figure 3. Facilitatory interactions in NTS

Intracellular recording from NTS neurone (membrane potential, −50 mV). *A*, response to electrical stimulation of the SN (2 pulses, 0·1 ms, 1 kHz, 8 V, given at 1 Hz). *B*, the stimulus, as in *A*, is preceded by stimulation of the HDA (5 pulses, 0·1 ms, 500 Hz, 100 μA, given at 1 Hz). In *A* and *B* 2 traces are superimposed. *C*, response of neurone to aortic balloon distension (Barotest) and response to 0·1 ml of 0·1 % sodium cyanide injected into the carotid artery (Chemotest; *D*). In these records the upper trace is the neuronal activity and lower trace is arterial blood pressure.

of excitation was evident, although more complex interactions were clearly possible and were particularly prevalent in those cases where evidence of chemoreceptor and baroreceptor convergence were obtained.

DISCUSSION

The material presented in this paper shows that, aside from exerting an indirect facilitatory influence on some NTS neurones that are excited on chemoreceptor stimulation as first reported by Silva-Carvalho *et al.* (1993), HDA stimulation can elicit a direct excitatory synaptic action on at least a proportion of NTS neurones that are also excited by inputs from the arterial chemoreceptors. Indeed, there may be cases where both facilitation and direct synaptic control occur; that is in those NTS neurones that are excited polysynaptically from the SN. This is the first demonstration of the direct excitatory input from the hypothalamus to 'chemosensitive' neurones of the NTS. The present data indicate that this input may be directed to NTS neurones that are monosynaptically excited on SN stimulation, but to others also. The former neurones would presumably provide the indirect facilitatory influences seen in many NTS neurones when the HDA is stimulated (see

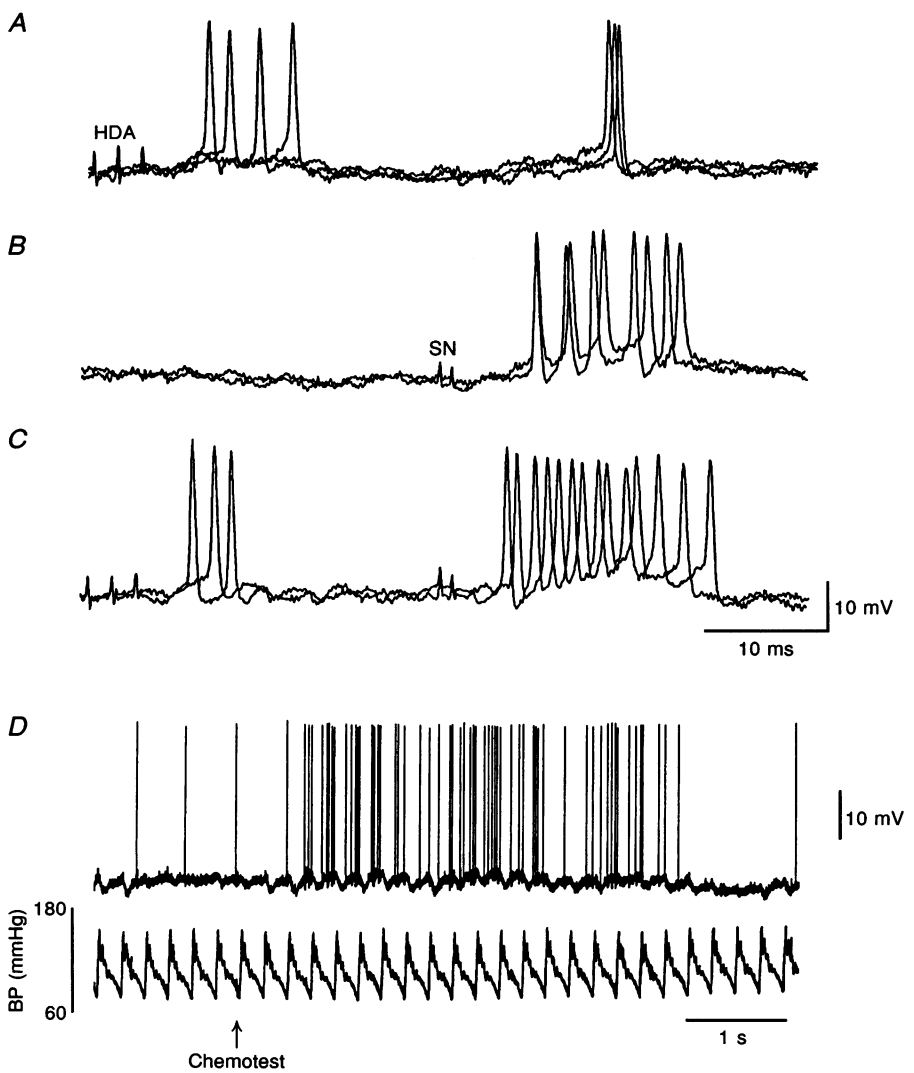

Figure 4. Powerful facilitation of chemoresponse on HDA stimulation

Intracellular recording from NTS neurone (membrane potential, −50 mV). *A*, stimulation of the HDA (3 pulses at 500 Hz; 0·1 ms, 50 μA, given at 1 Hz) evoked an early (8 ms latency) and late (35 ms latency) response. *B*, sinus nerve (SN) stimulation (2 pulses, 1 kHz, 0·1 ms, 5 V, given at 1 Hz) evokes a powerful synaptic response. *C*, simultaneous stimulation of HDA and SN (parameters as in *A* and *B*) evokes a more marked response. In *A* 3 traces and in *B* and *C* 2 traces are superimposed. *D*, response of this neurone to an intracarotid injection of 0·1 ml of 0·1 % sodium cyanide (Chemotest). Upper trace, neuronal discharge; lower trace, arterial blood pressure.

for example Fig. 3) and as described earlier (Silva-Carvalho *et al.* 1993). These patterns of response, and the intranuclear substrate that accounts for them (see Silva-Carvalho *et al.* 1995), can be expected to play a role in the mutual facilitatory interactions that result in enhanced cardiovascular and respiratory responses when these two inputs are activated (and see Dawid-Milner *et al.* 1995 for discussion). Furthermore, even more complex interactions may be possible, since both SN and HDA stimulation are able to elicit complex synaptic responses in some NTS neurones. A particular example is those NTS neurones in which SN stimulation evokes a response consisting of an EPSP–IPSP, in which the latter component is the result of an inhibitory input arising from the activation of baroreceptor afferents, whilst the former is an excitatory action of the chemoreceptors.

The 'chemosensitive' neurones identified in the present study were encountered over a more extensive area of the NTS than described in a recent study (Spyer *et al.* 1990) conforming more to the data provided by Mifflin (1993). These neurones, all of which failed to exhibit respiratory-related patterns of activity or to be modulated by lung inflation, would be expected to contribute to the chemoreceptor-evoked changes in respiration and the cardiovascular system. It is interesting to recall that the direct effects of chemoreceptor activation on respiratory neurones of the ventrolateral NTS have been shown to be inhibitory, the characteristic increase in activity arising in consort with the evoked enhancement of central respiratory drive (Lipski, McAllen & Spyer, 1977). This could imply that the actions of these 'chemosensitive' neurones in respiratory control are mediated by their connections beyond the NTS.

Few NTS neurones in the present study gave indications of receiving a convergence of baroreceptor and chemoreceptor inputs. Only three of the eighteen neurones investigated (17%) showed excitation in response to chemoreceptor stimulation and inhibition in response to baroreceptor activation, which is a significantly smaller proportion than described in a previous extracellular recording study, where 44% of neurones showed this pattern of convergence (Mifflin, 1993). However, baroreceptor influences were not tested exhaustively in the present experiments, emphasis being placed on selecting neurones for further investigation that, whilst activated by chemoreceptor inputs, had no excitatory baroreceptor input. Only four of the neurones studied (22%) were seen to respond to SN stimulation with an EPSP–IPSP; most chemosensitive neurones responded with EPSPs that were of short latency, compatible with monosynaptic activation. The effects of HDA stimulation were also often of short latency, but the evoked effects were often observed for up to 80 ms. This is, however, considerably shorter than the duration of IPSPs that are elicited in barosensitive (and SLN-activated) neurones of the NTS that may last for between 150 and 300 ms on HDA stimulation (Mifflin *et al.* 1988; Jordan *et al.* 1988; Silva-Carvalho *et al.* 1995; Dawid-Milner *et al.* 1995).

Neurones exhibiting a convergence of baroreceptor (inhibitory), chemoreceptor and HDA (excitatory) inputs clearly have physiological properties compatible with a role in the regulation of the sympathetic outflow to heart and blood vessels (see Fig. 5*B*). Mifflin (1993) argued this forcibly in the case of NTS neurones receiving such baroreceptor and chemoreceptor inputs. These neurones would not, however, be expected to be involved in the pathways that control the activity of cardiac vagal motoneurones, since these are excited by both the baroreceptors and chemoreceptors, and by SLN inputs (McAllen & Spyer, 1978; Spyer, 1981, 1990, 1994; Daly, 1984), whilst HDA stimulation induces an inhibition of these neurones (Spyer, 1984, 1994). The latter effect is a consequence of both a direct GABA-mediated action within the nucleus ambiguus (NA) and disfacilitation through HDA-evoked effects within the NTS reducing the effectiveness of baroreceptor inputs (see Spyer, 1984, 1990, 1994 for discussion). As yet there are no convincing data regarding the identification of an appropriate class of NTS neurone to mediate a direct pathway conveying excitatory baroreceptor and chemoreceptor effects, although Lipski, McAllen & Trzebski (1976) describe neurones in the vicinity of the NTS that were excited by both baroreceptor and chemoreceptor inputs (see also Fig. 5*B*). There is anatomical evidence supporting the view that NTS neurones make direct synaptic contacts with vagal preganglionic motoneurones in the NA (Deuchars & Izzo, 1991; Spyer, Brooks & Izzo, 1994) and the baroreceptor input to cardiac vagal motoneurones has been shown to be their major excitatory input (McAllen & Spyer, 1978).

The present results are particularly important in explaining the basis for the reciprocal actions of the baroreceptors and chemoreceptors in the control of sympathetic efferent activity. Neurones with excitatory inputs from the chemoreceptors and HDA, and inhibitory inputs from the arterial baroreceptors ('integrative' neurones), would provide important information to the brainstem circuits that regulate sympathetic discharge. HDA effects would be mediated in this case both through the excitatory input to chemosensitive neurones (see Fig. 5*A*) and, in the case of those that are disynaptically activated by the chemoreceptors, through facilitatory connections within the NTS, but also by a reduction in the effectiveness of baroreceptor inputs (Mifflin *et al.* 1988). Baroreceptor-mediated inhibition is always disynaptic, since it depends on the activation of a component of the GABA-containing neurones of the NTS making these 'integrative' neurones exquisitely sensitive to changes in both reflex input and central descending control (see Fig. 5*B*). The biophysical characteristics of these neurones

will be an additional factor and *in vivo* studies are required to provide an adequate description of the properties of this class of NTS neurone to allow comparison with the classifications that have been provided from *in vitro* studies (see Paton, Rogers & Schwaber, 1991; Paton, Foster & Schwaber, 1993). An example is the fact that in some neurones the after-hyperpolarizations accompanying an action potential suppress evoked excitatory inputs (see Fig. 2). This has been described as a feature of a significant proportion of NTS neurones *in vitro* (Felder & Mifflin, 1994) and may account for often prolonged periods of post-activation depression (Lipski & Trzebski, 1975; Felder, 1986). Equally their projections and connections both beyond and within the NTS are of considerable importance and are currently unresolved. It is, however, true that evidence exists to suggest that the sympatho-excitatory neurones of the rostroventrolateral medulla (RVLM) receive baroreceptor and chemoreceptor inputs over independent pathways (see Guyenet, 1990 for discussion) rather than receiving an excitatory input determined by the interplay of these two inputs at an antecedent level within the NTS. However, no data exist to negate such an action of NTS 'integrative' interneurones, since their effects could be mediated via alternative pathways within the CNS that act in addition to those involving the RVLM in modulating sympathetic activity (Spyer, 1990) or they could provide a proportion of the input that is received by the RVLM.

In conclusion, the present study has substantiated a major role for the NTS, not merely in the transmission of the

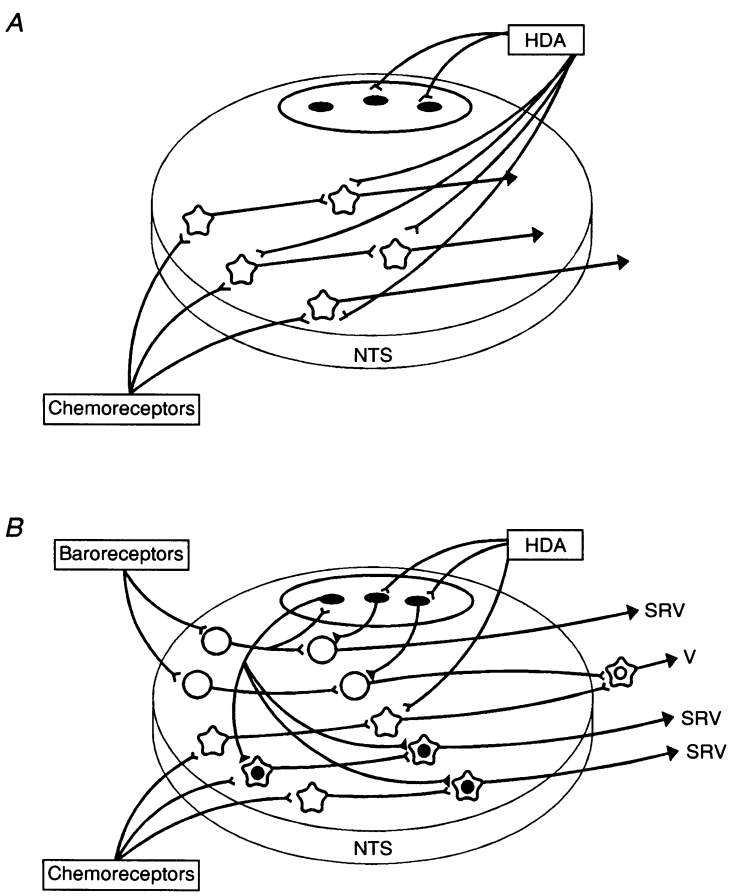

Figure 5. Schemes of NTS interactions

Schematic diagram of the connections within the nucleus tractus solitarii (NTS) that mediate arterial chemoreceptor inputs and interaction with the arterial baroreceptors. *A*, basic scheme of NTS connections mediating chemoreceptor reflex excitatory connections, shown as ➶. Intrinsic GABA-containing neurones are shown as filled ovals. Descending inputs from the HDA impinge on these, and NTS neurones, which receive, directly or indirectly, input from the arterial chemoreceptors (further details in the text). *B*, inputs to NTS from baroreceptors, chemoreceptors and HDA are shown. Exclusively baroreceptor-sensitive neurones are shown as ○; chemoreceptors are ⬠. Excitatory inputs are shown as ➶ and inhibitory as ➷ . Neurones receiving convergent baroreceptor and chemoreceptor inputs are shown as combined symbols: ⬟ when baroreceptor influence is excitatory, ⬟ when inhibitory. Further details in the text. R, respiratory; S, sympathetic; V, vagal.

arterial chemoreceptor reflex but also in its integration with other reflex inputs and with descending inputs concerned with broader aspects of cardiorespiratory and behavioural control. Whilst these observations have considerable physiological importance, they may also be significant for long-term changes in cardiovascular homeostasis. There are suggestions in the literature (Hilton, 1980) that an upregulation of the chemoreceptor reflex may participate in the aetiology of essential hypertension. A modification of the sensitivity of the synaptic mechanisms occurring within the NTS that have been revealed in this report may well contribute to this process.

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