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BARORECEPTOR INPUTS TO THE NUCLEUS TRACTUS SOLITARIUS IN THE CAT: MODULATION BY THE HYPOTHALAMUS

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SUMMARY

1. The effects of stimulation within the hypothalamic defence area (HDA) on the activity of neurones in the nucleus of the solitary tract (NTS) have been investigated *in vivo*.

2. HDA stimulation exerted marked influences on NTS neurones. Approximately two-thirds of units receiving a carotid sinus nerve (SN) input were inhibited by HDA stimulation. *All* units shown to receive an excitatory input from carotid sinus baroreceptors were inhibited by HDA stimulation.

3. The specificity of the HDA stimulation was investigated by generalized hypothalamic stimulation. In these experiments the number of units activated by SN stimulation that were inhibited was reduced considerably. A much smaller percentage (27%) of baroreceptive units were inhibited from hypothalamic stimulation outside the defence area.

4. Intracellular recordings revealed that HDA stimulation evoked a long-lasting hyperpolarization of membrane potential that resulted from postsynaptic inhibition (rather than disfacilitation). The HDA-evoked IPSP 'shunted' the SN-evoked EPSP when the SN stimulus was timed to occur during the initial peak hyperpolarizing phase of the HDA-evoked IPSP.

5. HDA stimulation disinhibited cells receiving an inhibitory input from the carotid sinus baroreceptors.

6. The effects of HDA stimulation were not limited to cells receiving SN afferent information or to cells within the NTS.

7. Our results explain, at the intracellular level, the mechanism for the central suppression of the baroreceptor reflex that forms an integral part of the defence response in the cat.

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INTRODUCTION

In a recent study in the rabbit, electrophysiological evidence has been provided for a convergence of inputs onto a limited number of NTS (nucleus of the solitary tract) neurones from the aortic nerve (AN) (which is solely baroreceptive in that species) and the central nucleus of the amygdala (CEN) (Cox, Jordan, Moruzzi, Schwaber, Spyer & Turner, 1986). It was suggested in that paper that this represented a mechanism whereby the autonomic, and specifically cardiovascular, component of the behavioural response that may be evoked from the CEN is expressed. Conversely, in the cat stimulating in the CEN (Timms, 1981), and also in the hypothalamic defence area (HDA), elicits a pattern of response that includes a central suppression of the baroreceptor reflex (Coote, Hilton & Perez-Gonzalez, 1979). There are indications in the literature (Adair & Manning, 1975; McAllen, 1976) that this results from an inhibitory action of hypothalamic stimulation on transmission through the nucleus of the solitary tract. However, there is evidence against presynaptic inhibition of sinus nerve (SN) afferent terminals occurring during HDA stimulation, (Jordan & Spyer, 1979), but the precise synaptic action of the hypothalamus exerted at the level of the NTS has not been studied in any detail.

Accordingly we have used both extra- and intracellular recordings from NTS neurones during stimulation of points within the HDA and elsewhere, and both SN and specific baroreceptor stimulation to determine the nature of the synaptic interaction of these inputs. Preliminary reports of some aspects of this study have been presented (Mifflin, Spyer & Withington-Wray, 1986; Spyer, Mifflin & Withington-Wray, 1987*b*).

METHODS

These experiments were carried out using the forty-five animals described in the preceding paper (Mifflin, Spyer & Withington-Wray, 1988) in which anaesthesia was induced (30–40 mg /kg I.V.) and maintained (i.e. 1–3 mg/kg as required) using sodium pentobarbitone. Under this anaesthestic regime, SN stimulation consistently elicited a depressor response whilst under chloralose anaesthesia it most often evoked a pressor response, as previously described (Neil, Redwood & Schweitzer, 1949). In identifying the HDA it was essential to be able to observe hypothalamic influences on the depressor component of the baroreceptor reflex as described by Coote *et al.* (1979).

The details of both the surgical preparation and recording arrangement were as described previously (Mifflin *et al.* 1988). In addition, using stereotaxic co-ordinates, a concentric bipolar stimulating electrode was positioned within the perifornical region of the hypothalamus between 10–12 mm anterior and 2–5 mm below stereotaxic zero (Berman, 1968) and 1·5–2·0 mm lateral to mid-line. To locate the defence area, (see Results), the hypothalamus was stimulated at 70 Hz with 1 ms pulses at 50–300 μA. Stimulation sites were marked at the end of the experiment by passing 500 μA DC current for 10–15 s and the stimulation site verified histologically.

RESULTS

Localization of hypothalamic stimulation site

The hypothalamic 'defence area' (HDA) was identified by observing the autonomic, and particularly the cardiovascular responses, to stimulation using a stereotaxically orientated bipolar microelectrode. On the basis of the criteria of

Coote *et al.* (1979), that stimulation within the HDA will evoke a sustained increase in arterial and pulse pressure, retraction of the nictatating membrane, pupillodilatation, and occasionally piloerection together with a block of the depressor response elicited by either electrical stimulation of the sinus nerve (SN) or increases in pressure within the carotid sinus, stimulation sites were localized as shown in Fig.

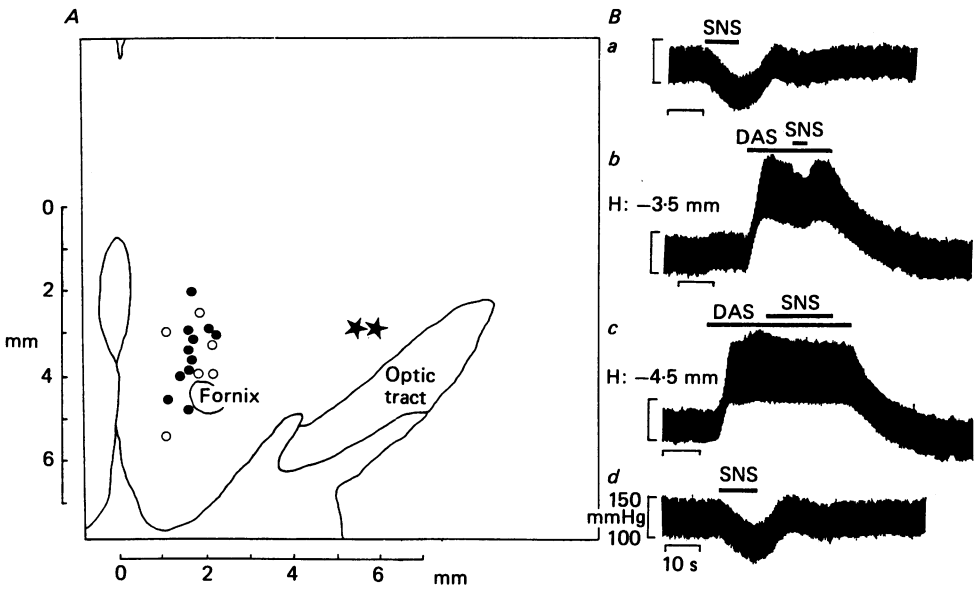

Fig. 1. *A*, hypothalamic stimulation sites 10–11 mm anterior to stereotaxic zero from which the defence reaction was evoked (●) and sites which evoked a comparable increase in mean and pulse pressure but did not inhibit the carotid sinus depressor reflex (○). Each point from a different animal. The stars represent stimulation sites within the amygdalo-hypothalamic pathway which evoked the defence reaction. *Ba*, blood pressure response to electrical stimulation of the carotid sinus nerve (SNS). *b*, response to hypothalamic stimulation (DAS) at site which did not block the sinus nerve depressor reflex. *c*, stimulation evokes comparable increases in mean and pulse pressure as in *b* but the sinus nerve depressor reflex was abolished. *d*, SNS alone still evoked a depressor reflex. Bars over blood pressure traces indicate periods of stimulation.

1*A* and *Bc*. There were also points in the near vicinity from which all features other than the blockade of the sinus reflex could be observed and these are illustrated in Fig. 1*A* and *Bb*. We also confirmed that stimulating with identical parameters in the amygdalo-fugal pathway (Timms, 1981), as it crosses over the optic tract, evoked the full pattern of the defence reaction including a suppression of the baroreceptor reflex (see Fig. 1).

Extracellular studies in the NTS

We recorded extracellularly from 179 units which were activated by electrical stimulation of the SN. Subsequently, the response to inflation of a balloon-tipped catheter placed within the ipsilateral SN was tested in ninety-eight of these neurones with thirty-one being activated (Fig. 2*B*). After such characterization of the unit, a

conditioning–test paradigm was used to examine the effects of a defence area stimulus on SN-evoked activity. Two pulses to the HDA at 500 Hz evoked a slight dilatation of the pupils whilst during a 30 ms burst the pupillary dilatation was maximally developed. Therefore conditioning bursts of HDA stimulation of 30 ms, 500 Hz were used. As long as no more than two of these 30 ms bursts of defence area stimuli were presented at a frequency of less than 0·5 Hz there was either no change or only a slight increase in arterial pressure.

Figure 2 illustrates the effects of HDA stimulation on a neurone activated by both SN stimulation and baroreceptor activation. Preceding the SN stimulus by 10 ms with a 30 ms burst of HDA stimulation completely abolished the discharge evoked by SN stimulation (Fig. 2*A*). Increasing the interval between the HDA and SN stimuli revealed that discharge was inhibited for between 210 and 310 ms. In addition to its influence on evoked discharge, HDA stimulation also abolished the ongoing activity of this particular unit for approximately 150 ms (Fig. 2*C*).

Approximately two-thirds of units receiving an SN input, ($n = 118$) whether this was the sole input or accompanied by other convergent inputs, were inhibited by HDA stimulation. Without exception, every unit receiving an excitatory input from the carotid sinus baroreceptors ($n = 31$, nineteen with an exclusive SN input, twelve with convergent inputs) was inhibited by HDA stimulation. Discharge evoked by nerve stimulation was abolished or markedly reduced when this followed a burst of HDA stimulation at conditioning–test intervals of 10–50 ms. In twelve neurones tested the inhibition persisted for conditioning intervals of 150–300 ms. Increasing the duration of the HDA conditioning train from 30 to 130 ms (i.e. fifteen to sixty-five pulses) did not further increase the degree or duration of the inhibition. Spontaneous discharge and convergent inputs, when present, were similarly inhibited following HDA stimulation. We never observed, within the approximate boundaries of the NTS, HDA inhibition of one excitatory input and not another. In only three neurones did HDA stimulation initially evoke a brief discharge (prior to the onset of the inhibition).

In 29% (thirty-seven neurones) of units receiving an exclusive SN input and 25% (thirteen neurones) of those receiving convergent inputs evoked and spontaneous discharge were not altered by HDA stimulation. In those cells unaffected by HDA stimulation, increases in the duration and/or frequency of the stimulus train and/or stimulation intensity (up to 1 mA) did not produce an inhibition of discharge. In a small number of cells with a varying pattern of afferent input ($n = 11$), HDA stimulation evoked discharge orthodromically (variable onset latencies, failure to collide with spontaneous or evoked spikes) with latencies of 6·4–17·9 ms. This HDA excitatory input was capable of summating with the SN excitatory input when both were stimulated simultaneously. It is notable that whilst a larger proportion of cells that received a SN input were affected by HDA stimulation, only a relatively small number of neurones receiving an exclusive vagal (VN) input ($n = 24$) received either excitatory ($n = 2$; 8%), inhibitory ($n = 6$; 25%) or no ($n = 16$; 67%) inputs from the HDA.

Effects of stimulating outside of the HDA

To determine whether the effects of HDA stimulation were restricted to activating highly localized neural elements, in five cats the hypothalamic electrode was

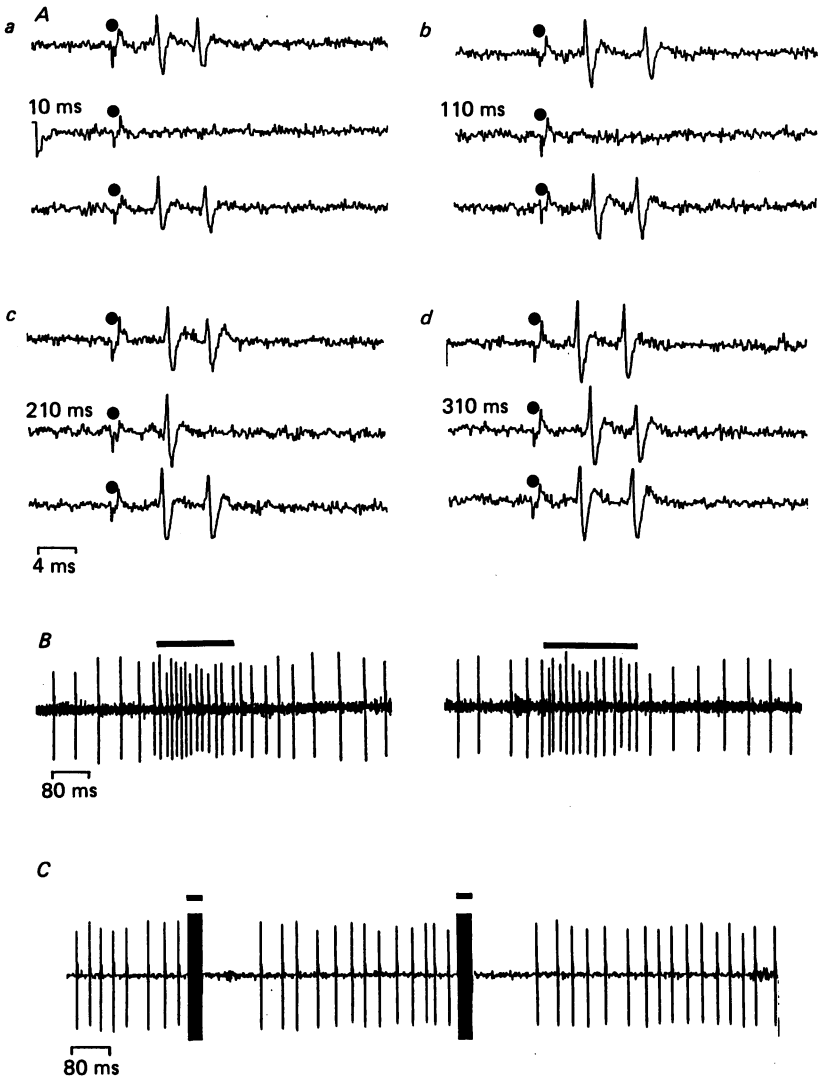

Fig. 2. Extracellularly recorded effects of defence area stimulation (HDAS). *A a–d*, top traces: discharge evoked by electrical stimulation of the SN (stimulus artifact indicated by ●). Middle traces: response to SN stimulation when preceded by 30 ms, 500 Hz, train of HDAS. The numbers are the interval between the end of the HDAS and the SN stimulus. Bottom traces: SN stimulation in next stimulus cycle after the HDAS. *B*, responses to inflation of intra-sinus balloon during periods indicated by the bars. *C*, inhibition of spontaneous discharge following HDAS.

positioned at a site which evoked a sustained pressor response but did not inhibit the carotid sinus depressor reflex (e.g. the open points in Fig. 1*A*, the response illustrated in Fig. 1*Bb*). In all categories the vast majority of units ($n = 29/32$; 91%) were unaffected by hypothalamic stimulation at sites that did not conform to our definition of the HDA. Whilst HDA stimulation invariably inhibited units receiving excitatory baroreceptor inputs, this was not the case when stimulating outside of the

HDA. Here, stimulation inhibited only 27% ($n = 3/11$) of the cells excited by baroreceptor activation which received an exclusive SN input or convergent inputs. Experiments where the hypothalamus was stimulated at points outside of the HDA were the only instances where we observed baroreceptor-activated units which were not inhibited by hypothalamic stimulation.

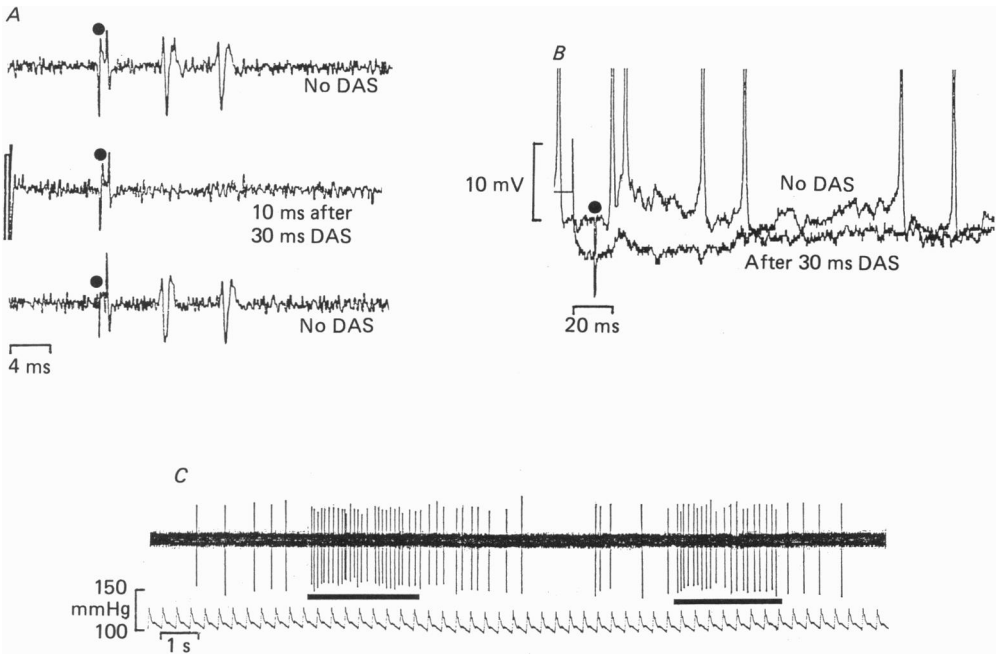

Fig. 3. Extracellular and intracellular recording of HDA inhibition of a cell excited by activation of carotid sinus baroreceptors. *C*, extracellular recording of response to baroreceptor activation (during period indicated by the bars). *A*, extracellular responses to SN stimulation (stimulus artifact indicated by ●) in top and bottom traces. Between the two SN stimuli the SN stimulus was preceded by 10 ms by a 30 ms burst of HDA stimulation (middle trace) which abolished the SN-evoked discharge. *B*, intracellular recording from same cell. SN stimulation alone (top trace – no defence area stimulation, DAS) evoked a comparable number of action potentials (truncated) at the same latency as recorded extracellularly. At the end of HDA stimulation (lower trace – after 30 ms DAS) membrane potential was hyperpolarized (for approximately 150 ms) and SN stimulation (10 ms after the end of DAS) did not evoke discharge. Membrane potential: −53 mV. Potassium citrate-filled electrode.

Intracellular studies

With extracellular recordings it is not possible to discern whether the inhibition of SN-evoked discharge following HDA stimulation resulted from pre-synaptic inhibition (primary afferent depolarization), disfacilitation or postsynaptic inhibition. To resolve this issue intracellular recordings were undertaken.

In the case of seventeen neurones that were impaled following an extracellular characterization of the HDA-evoked inhibition of the SN input, intracellular recordings revealed that HDA stimulation evoked a long-lasting hyperpolarization of membrane potential (Fig. 3). The hyperpolarization developed during the 30 ms

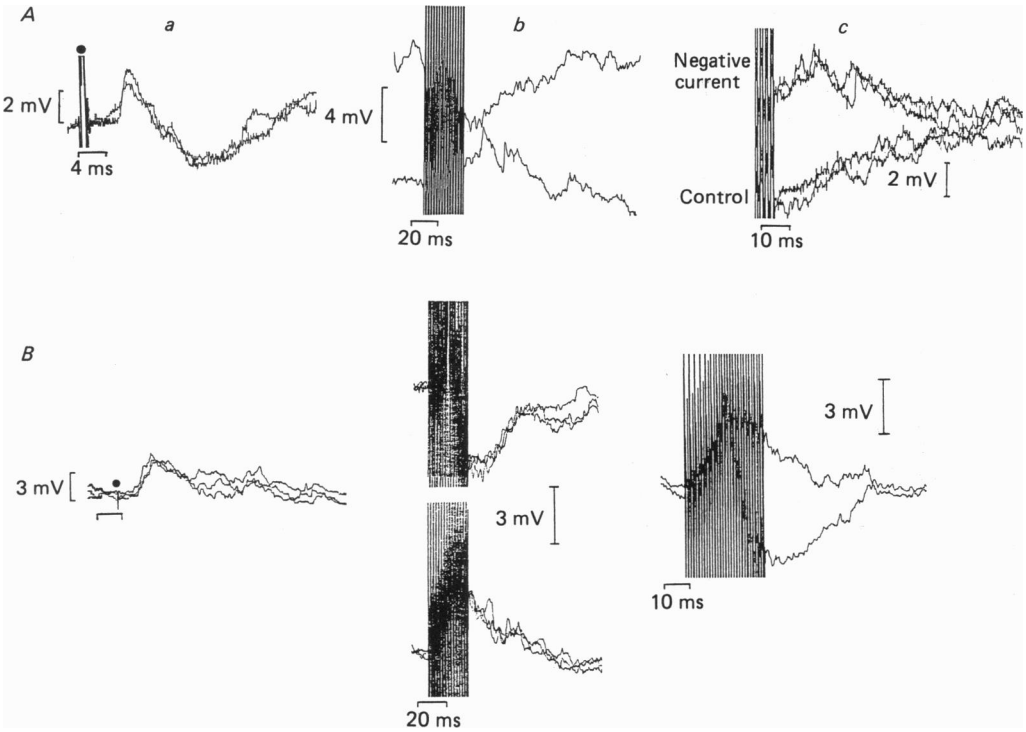

Fig. 4. Reversal of polarity of hyperpolarization evoked by HDA (*A*) and amygdalo-hypothalamic pathway (*B*) stimulation in two cells during passage of hyperpolarizing DC current. Potassium citrate-filled electrodes. *Aa* and *Ba*, response of cells to SN stimulation. *Aa*, two sweeps of EPSP–IPSP superimposed, membrane potential = −52 mV. *Ba*, three sweeps of EPSP superimposed, membrane potential = −56 mV. *Ab* and *Bb*, response to 30 ms, 500 Hz hypothalamic stimulation under control conditions (top trace) and during passage of hyperpolarizing DC current (bottom trace) (4 nA in *Ab*; 6 nA in *Bb*). *Ab*, one sweep each. *Bb*, three sweeps each superimposed. Traces in *Ab* and *Bb* are separated by an arbitrary DC level to aid visualization. *Ac* and *Bc*, faster sweep speed with reversed IPSP shifted above the control hyperpolarizing IPSP to permit comparison of time courses. *Ac*, two sweeps each superimposed. Initial 20 ms of HDA stimulation omitted. *Bc*, one sweep each. In *Bc* note, during the stimulus artifacts, that the amygdalo-hypothalamic pathway stimulation evoked an EPSP prior to the IPSP.

burst of HDA stimulation, the membrane being at its most hyperpolarized level at the cessation of the HDA stimulus. The SN-evoked EPSP was decreased in amplitude and duration during this evoked hyperpolarization and failed to depolarize the membrane to threshold for spike discharge.

To distinguish between disfacilitation and postsynaptic inhibition the effects of the passage of hyperpolarizing DC currents (4–12 nA) were examined on the HDA-evoked hyperpolarization. During application of hyperpolarizing DC current both the HDA-evoked hyperpolarization and the hyperpolarization evoked by stimulation of the amygdalo-hypothalamic pathway reversed in polarity to depolarizing potentials (Fig. 4), suggesting that they resulted from postsynaptic inhibition. The time course and shape of the reversed depolarizing response were not identical to

those of the control hyperpolarizing IPSP. It is noteworthy that stimulation of the amygdalo-hypothalamic pathway evoked an EPSP–IPSP sequence (eight of eleven cells) (Fig. 4*B*) whilst HDA stimulation evoked an IPSP. However, as described above, our extracellular recordings revealed that three of 118 cells receiving excitatory SN inputs which were inhibited by HDA stimulation had a short-latency initial excitation to HDA stimulation.

TABLE 1. HDA-evoked PSPs in NTS neurones influenced by SN stimulation

	Carotid SN EPSP	Carotid SN EPSP–IPSP	Carotid SN IPSP
Defence area input (*n*)	IPSP (34 of 41 tested)	IPSP (5 of 8 tested)	EPSP (3 of 6 tested)
Amplitude (mV)	4·1 ± 1·7 (1·2–7·7)	3·6 ± 1·6 (1·8–6·2)	1·6, 4·3, 6·9
Duration (ms)	129 ± 47 (68–245)	83 ± 32 (33–117)	37, 48, 57

Values are expressed as mean ± S.D. Defence area stimulated by 30 ms, 500 Hz train of 1 ms pulses at 50–300 μA.

TABLE 2. HDA-evoked IPSPs in cells tested for excitatory input from carotid sinus baroreceptors

	Baroreceptor excitation		No baroreceptor input
Number receiving DA IPSP	15 of 15 tested		11 of 14 tested
Amplitude of IPSP (mV)	4·5 ± 1·9	$P = 0·46$	4·0 ± 1·0
Duration of IPSP (ms)	132 ± 53	$P = 0·47$	118 ± 34

Values are expressed as mean ± S.D. Defence area stimulated by 30 ms, 500 Hz train of 1 ms pulses at 50–300 μA. DA, defence area.

Evidence that the HDA-evoked hyperpolarization was a chloride-dependent IPSP was obtained by comparing its properties in cells whose activity was recorded with potassium chloride- and potassium citrate-filled electrodes respectively. The amplitude of the evoked hyperpolarization was significantly greater ($P = 0·004$) in cells impaled with a potassium citrate-filled electrode ($n = 18$; mean ± S.D. = 5·1 ± 1·4 mV; range = 2·7–7·7 mV) than with a potassium chloride-filled electrode ($n = 16$; 2·8 ± 1·0 mV; 1·2–4·4 mV). Presumably, this difference resulted from chloride diffusion from the electrode lowering the chloride equilibrium potential and so reducing the amplitude of the IPSP. The duration of the IPSP was not different ($P = 0·06$) in cells impaled with potassium citrate ($n = 18$; 144 ± 51 ms; 77–245 ms) than with potassium chloride-filled electrodes ($n = 16$; 113 ± 38 ms; 68–200 ms).

Table 1 presents the amplitude and latency of the HDA-evoked IPSP in cells receiving an EPSP or an EPSP–IPSP from SN stimulation. There appeared no difference in the form of the HDA-evoked IPSP in cells excited by activation of the carotid sinus baroreceptors compared to cells receiving no apparent input from them (Table 2). Onset latencies of the HDA-evoked IPSP ranged from 10 to 30 ms.

Postsynaptic inhibition would be expected to have equivalent effects on other excitatory inputs to a cell. Our extracellular results reviewed above revealed no selective inhibition of one convergent excitatory input as opposed to another and this was confirmed by our intracellular recordings. As illustrated in Fig. 5, following HDA

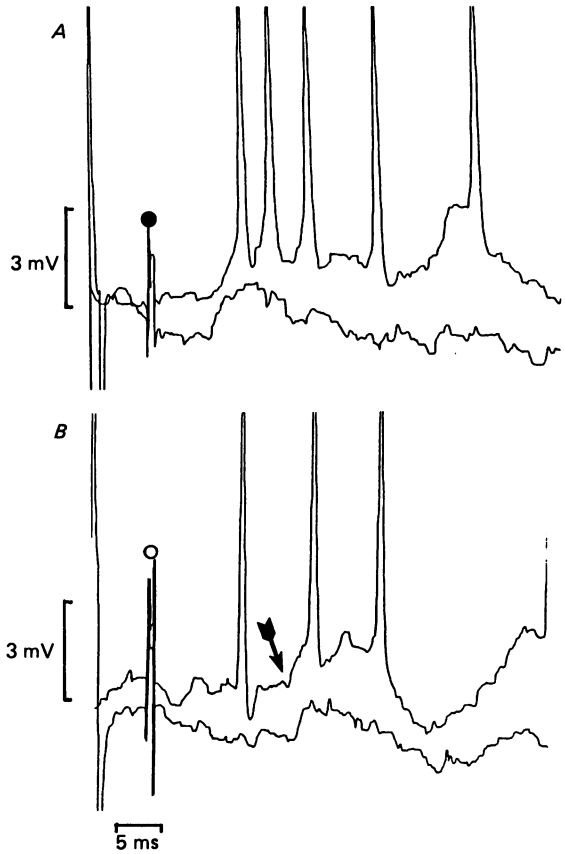

Fig. 5. Inhibition of SN (*A*, artifact indicated by ●) and vagal (*B*, artifact indicated by ○) inputs following HDA stimulation. Top traces in *A* and *B* are the responses to nerve stimulation alone and the lower traces are the responses following HDA stimulation. In *B*, in the upper 'control' trace, a spontaneous action potential preceded the vagally evoked EPSP, marked by the arrow. Action potentials are truncated. Membrane potential: −58 mV. Potassium citrate-filled electrode.

stimulation the membrane potential was hyperpolarized so that both SN and the longer-latency VN-evoked PSPs failed to evoke action potential discharge.

The nature of the evoked IPSP

We examined the effects of varying the HDA stimulation parameters of stimulation on the HDA-evoked IPSP. In no instance did a change in the number or frequency of HDA stimuli alter the nature of the response. HDA stimulation always evoked an IPSP but changes in stimulus parameters produced quantitative changes in the IPSP (e.g. change in onset latency, amplitude and duration).

Figure 6 illustrates the effects of varying the number of stimuli (at 500 Hz) to the HDA. In the cell depicted in Fig. 6*A*, two pulses evoked a comparatively small-amplitude, short-duration IPSP. Increasing the number of pulses to five markedly increased the amplitude and duration of the IPSP without affecting the onset

latency. A further increase of up to fifteen stimuli produced no further increase in amplitude but again increased the duration of the IPSP. Increasing the duration of the 500 Hz burst of HDA stimuli to 130 ms (sixty-five pulses) did not further prolong the duration or increase the amplitude of the HDA-evoked IPSP (not illustrated).

Interaction of SN-evoked EPSP and HDA IPSP

As illustrated in Figs 3 and 5, during an HDA-evoked IPSP SN-evoked EPSPs were markedly decreased in amplitude and duration. This 'shunting' was observed

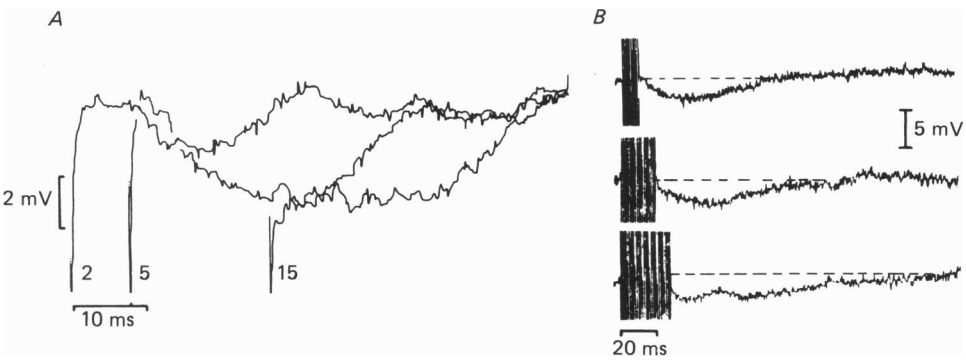

Fig. 6. Relationship between number of HDA stimuli and the amplitude and duration of the HDA-evoked IPSP. *A*, response to different numbers (two, five and fifteen) of HDA stimuli (at 500 Hz). Vertical lines to the left of each number mark the end of the HDA stimuli. The trace illustrating the response to five HDA stimuli was interrupted to differentiate it from the response to two pulses. Membrane potential: −52 mV. Potassium citrate-filled electrode. *B*, responses to 10, 20 and 30 ms burst of HDA stimulation. Traces separated by arbitrary DC amount. Membrane potential: −61 mV. Potassium chloride filled-electrode.

in all neurones in which the SN stimulus was timed to evoke an EPSP during the period of an HDA-evoked IPSP. For example, in Fig. 7*A* the comparatively short-latency (3·5 ms) SN-evoked EPSP was decreased in duration during the HDA IPSP but was still apparent. In the cell depicted in Fig. 7*B*, the longer-latency (9·5 ms) SN-evoked EPSP was absent during the HDA IPSP. In the latter case it is possible that the large HDA-evoked IPSP shunted the SN EPSP and/or that the SN EPSP did not reach the cell due to postsynaptic inhibition of antecedent cells receiving shorter-latency SN inputs. Thus the absence of an SN-evoked EPSP in Fig. 7*B* may result from disfacilitation although the cell was also postsynaptically inhibited by HDA stimulation.

This 'shunting' of SN-evoked EPSPs was observed only when the SN stimulus was timed to occur during the initial, peak hyperpolarizing phase of the HDA-evoked IPSP (Fig. 8). When the SN stimulus was presented so that the EPSP occurred during the declining phase of the IPSP, it was not shunted even though membrane potential remained slightly hyperpolarized. Note that at the peak of the HDA IPSP, whilst the SN EPSP was decreased in duration the latency and initial rise time appeared to be unaffected (Fig. 8*B*). Presumably these effects are dependent on the time course of the changes in membrane input resistance (R_n) accompanying the

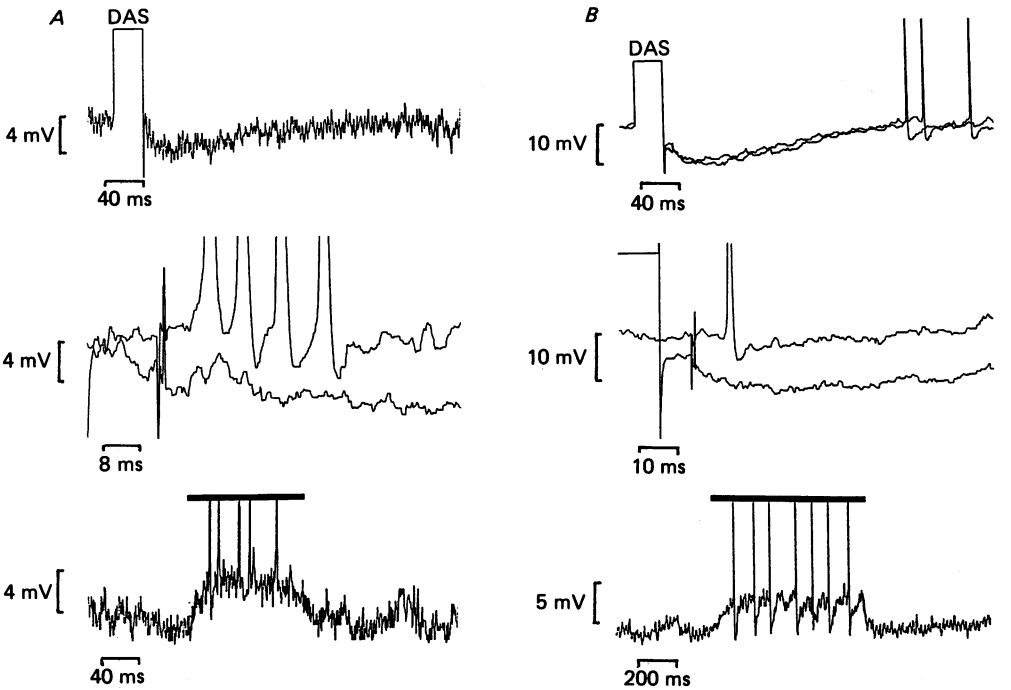

Fig. 7. SN-evoked EPSP during HDA-evoked IPSP in two cells, one (*A*) receiving a relatively short-latency (3·5 ms) and the other (*B*) a longer-latency (9·5 ms) SN input. Both cells were excited by activation of the carotid sinus baroreceptors (bars over the bottom traces – action potentials truncated). Top traces: examples of IPSP evoked by a 30 ms, 500 Hz burst of HDA stimulation (DAS). In *B* two sweeps are superimposed. Middle traces: upper sweep – response to SN stimulation in absence of DAS. Lower sweep – SN stimulation after cessation of DAS. Membrane potential: $A = -52$ mV; $B = -63$ mV. HRP-filled electrodes.

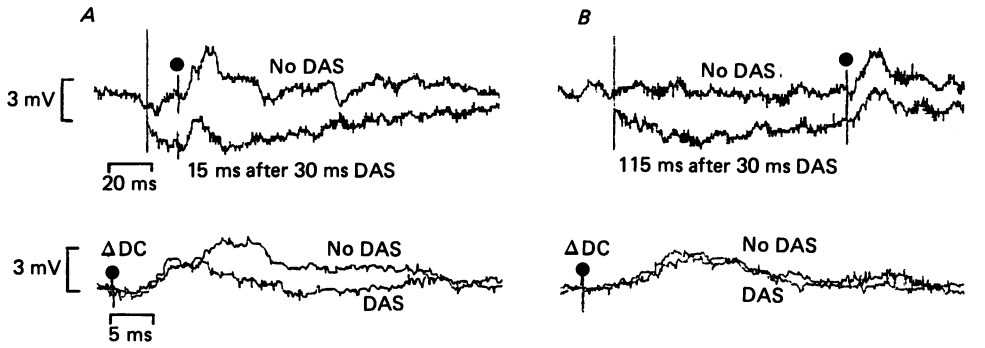

Fig. 8. SN-evoked EPSP at peak (*A*) and towards end (*B*) of HDA-evoked IPSP. In *A* the upper sweeps are the responses to SN stimulation (stimulus artifact indicated by ●) in the absence of HDA stimulation (DAS). Lower sweeps are responses to SN stimulation 15 (*A*) and 115 ms (*B*) after end of 30 ms, 500 Hz DAS. The vertical lines mark the end of the DAS. In *B* the SN-evoked EPSPs alone and after DAS are superimposed by shifting the DC level of the two sweeps. Membrane potential: −51 mV. Potassium citrate-filled electrode.

IPSP. Indeed, using constant-amplitude inward current pulses an average decrease in R_n was measured during the peak of the HDA IPSP of 15–43% ($n = 8$). This decreased R_n was not a result of membrane rectification as hyperpolarization of membrane potential to the same level as reached during the HDA IPSP did not decease R_n. This makes it clear that the decrease in R_n resulted from a conductance

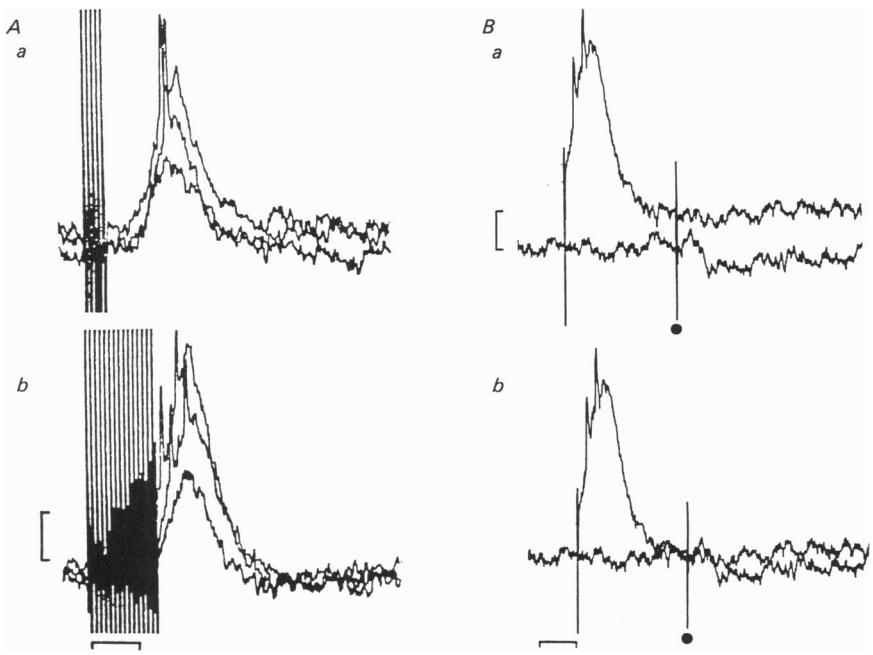

Fig. 9. Cell inhibited by SN stimulation and activation of carotid sinus baroreceptors and receiving an EPSP after HDA stimulation (HDAS) (same cell as in Fig. 7 of Mifflin, Jordan, Withington-Wray & Spyer, 1988). *A*, three sweeps superimposed of response to 10 (*Aa*) and 30 ms (*Ab*) 500 Hz burst of HDAS. *Ba*, response to SN stimulation (stimulus artifact indicated by ●) in the absence of HDAS (lower sweep) and following a 30 ms, 500 Hz burst of HDAS (upper sweep). DC level of traces shifted to aid visualization. *Bb*, sweeps in *Ba* with no shifting of DC level. HDA stimulus artifacts erased; vertical bar marks end of HDAS. Membrane potential: −61 mV. Potassium citrate electrode. Calibration bars: amplitude 4 mV; time 20 ms.

increase produced by the IPSP. Further, the shunting of the SN-evoked EPSP was not a result of any rectifying properties of the membrane as hyperpolarization of membrane potential to the level reached at the peak of the HDA IPSP did not decrease EPSP amplitude or duration, rather EPSP amplitude was increased.

HDA stimulation in cells inhibited by SN inputs

The effects of HDA stimulation on cells receiving inhibitory SN afferent, and specifically baroreceptor, inputs were particularly interesting and emphasize the profound influence of HDA stimulation on SN inputs. Of the cells in which SN stimulation evoked an IPSP, HDA stimulation evoked an EPSP in three of six cells tested with onset latencies of 7·4, 14·9 and 21·1 ms (Table 1).

HDA-evoked EPSPs, whilst constant in latency and duration, varied greatly in amplitude (Fig. 9*A*). The amplitude of the HDA-evoked EPSP was dependent

however upon the number of HDA stimuli. Increasing the number of pulses in each train increased the amplitude and decreased its onset latency but did not alter its duration.

An invariant effect of HDA stimulation on cells receiving inhibitory SN inputs was that following HDA stimulation (whether evoking an EPSP or not) the SN-evoked IPSP was either absent (Fig. 9*B*) or dramatically decreased in duration (Fig. 10*A*)

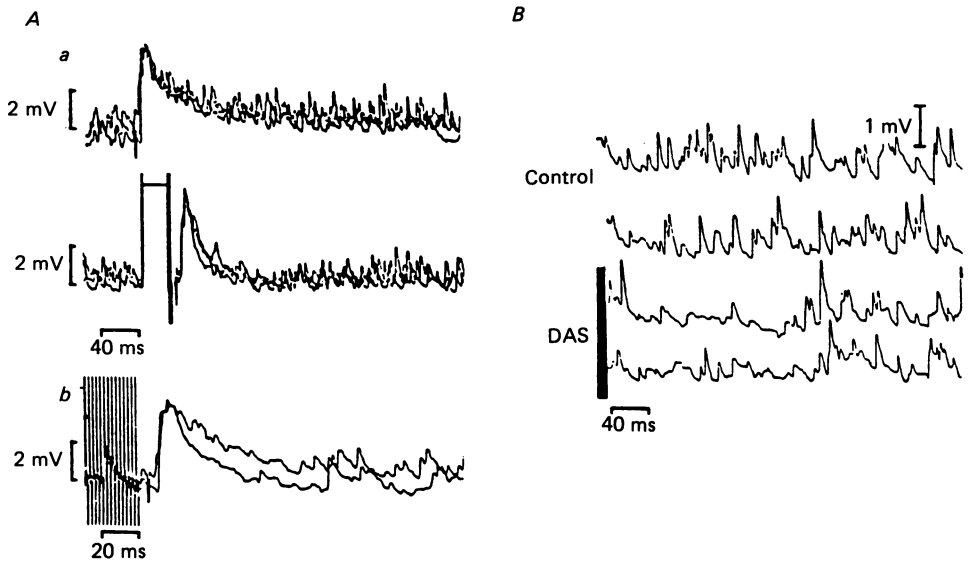

Fig. 10. Cell inhibited by SN stimulation and activation of carotid sinus baroreceptors and receiving no direct input from HDA (same cell as in Fig. 8 of Mifflin *et al.* 1988). *A a*, two sweeps of reversed SN-evoked IPSP alone (top) and following a 30 ms, 500 Hz burst of defence area stimulation (DAS; bottom). *A b*, one sweep of reversed SN IPSP alone superimposed with reversed IPSP after DAS. Note faster sweep speed compared to *A a*. *B*, top two traces are sweeps of membrane potential in the absence of DAS (control). Bottom two traces are sweeps of membrane potential after DAS. Note the reduction in amplitude of synaptic noise (reversed IPSPs). Traces separated by an arbitrary DC level to aid visualization. Vertical bars are the stimulus artifacts of the last 20 ms of the 30 ms burst of DAS.

with no change in membrane potential. In the cell depicted in Fig. 9*B* following HDA stimulation, and after the evoked EPSP, the SN-evoked IPSP was absent, SN stimulation now producing no change in membrane potential. In the cell depicted in Fig. 10*A*, HDA stimulation did not evoke an EPSP or any change in membrane potential. However, the duration of the SN-evoked IPSP (reversed in polarity to a depolarizing potential due to an increased intracellular chloride concentration) was dramatically reduced with no change in the peak amplitude of the reversed IPSP. In this cell the spontaneous synaptic noise (reversed IPSPs) was markedly decreased in amplitude following HDA stimulation (Fig. 10*B*) indicating tonic inhibitory inputs were reduced.

Therefore, in cells receiving inhibitory SN inputs, HDA stimulation disinhibited the cells as, following HDA stimulation, SN stimulation either no longer evoked an

IPSP (Fig. 9) or evoked an IPSP of greatly decreased duration (Fig. 10). As the HDA effects on the SN-evoked IPSP were observed in the absence of any change in membrane potential we infer the disinhibition was produced by the HDA-evoked postsynaptic inhibition of cells excited by SN stimulation and antecedent to the inhibited cell. Also, the fact that the SN-evoked IPSP in Fig. 10 was decreased in duration but not amplitude indicates that under certain circumstances either polysynaptic or slower conducting inputs were blocked or the disinhibition preferentially removes distally located inhibitory inputs.

Specificity of HDA effects

The effects of HDA stimulation were not limited to cells receiving SN afferent inputs or to cells within the NTS. The spontaneous activity of neurones receiving cutaneous afferent inputs in the dorsal column nuclei was either enhanced or inhibited by HDA stimulation. Also, SN inputs to reticular units, which were clearly outside the boundaries of the NTS, were often inhibited by HDA stimulation. Within the vagal dorsal motor nucleus, the pattern of evoked inhibition differed as stimulation was capable of inhibiting one afferent input compared to another. HDA stimulation was observed to selectively suppress SN inputs but did not alter anti- or orthodromic vagal inputs.

DISCUSSION

The results of the present study indicate that activation of the defence area of the hypothalamus exerts profound influences on neurones located within, and in the immediate vicinity of, the NTS. In the context of the cardiovascular component of the defence response in the cat, this influence can explain the central suppression of the baroreceptor reflex – both cardiac and vasorepressor components – that form an integral feature of the response. Moreover, as a distinct pattern of descending influence has been identified that is closely correlated to the action of peripheral afferent inputs, and particularly that of the baroreceptors, on these NTS neurones, our observations allow speculations about the nature of the neural circuitry within the NTS that is responsible for the integration of these inputs. Further, it is possible to extrapolate from these suggestions to the role of the NTS in cardiovascular homeostasis.

Since 1963, it has been established that electrical stimulation within a restricted region of the hypothalamus can suppress the baroreceptor reflex through an action within the CNS (Hilton, 1963; Coote *et al.* 1979). The synaptic basis of this action has remained, until now, unresolved. More recently, however, the role of the hypothalamus in integrating the behavioural and cardiovascular components of the alerting response has been brought into question by the inability to elicit such a pattern of response on stimulating chemically within the hypothalamus (Hilton & Redfern, 1986). This implies that electrical stimulation is activating fibres of passage, perhaps a pathway descending from the central nucleus of the amygdala which has an important role in affective behaviour including its cardiovascular component (Timms, 1981). Indeed, we were able to evoke such a pattern of response, including a suppression of the baroreceptor reflex, on stimulating the functional pathway

between the amygdala and hypothalamus as it courses over the optic tract. In our experience, and as reported by Coote *et al.* (1979), the full expression of response on electrical stimulation in the medial hypothalamus is evoked from a highly localized region, although components other than suppression of the baroreceptor reflex can be elicited from stimulation over a wide area. Intuitively this suggests that the perifornical region is more than just a fibre passage and is likely to represent an integrative area. Whatever, in the context of the pattern of response that is evoked on hypothalamic stimulation, our observations are valid whether or not it is a site of integration since we are concerned with the physiological expression of a pattern of behavioural response.

The inhibitory, and excitatory, influences of such hypothalamic stimulation are not restricted to those neurones of the NTS that are affected by SN, AN or other peripheral afferent inputs. Respiratory neurones of the lower brain stem (Ballantyne, Jordan, Spyer & Wood, 1986), reticular neurones and hypoglossal motoneurones (S. W. Mifflin, K. M. Spyer & D. J. Withington-Wray, unpublished observations), are also affected. Additionally, cardio-inhibitory vagal motoneurones located in the n. ambiguus are powerfully inhibited (Spyer, 1984; Spyer & Jordan, 1987). It is, however, clear that *all* NTS neurones that are excited by specific activation of the carotid sinus baroreceptors are inhibited by electrical stimulation of this restricted region of the hypothalamus (and in the amygdalo-fugal pathway). Such an influence on 'baroreceptor-sensitive' NTS neurones is similar to that reported by Adair & Manning (1975) and McAllen (1976). They both demonstrated that stimulation within the hypothalamus could inhibit NTS neurones receiving excitatory inputs from the SN, and McAllen (1976) went further to show that baroreceptor inputs were similarly affected. Adair & Manning (1975) failed to confirm that their stimulus protocol actually inhibited the vasodepressor component of the baroreceptor reflex, whilst McAllen (1976) thoroughly characterized his sites of stimulation. In this respect we have shown in the present study that on stimulating at sites in the hypothalamus which did not block the vasodepressor response, whilst they might affect the cardiac component, only 27% of NTS neurones receiving baroreceptor inputs were inhibited. This contrasts with the inevitable inhibition whenever the electrode was positioned at a site classified as the 'defence area'.

The original observations of McAllen (1976), which are directly comparable to those presented in this report, were limited to extracellular recordings from NTS neurones. Accordingly it was not possible to distinguish between an effect evoked by postsynaptic inhibition, presynaptic inhibition, disfacilitation or a mixture of these processes. Presynaptic inhibition at the level of the primary afferent terminals of the baroreceptor afferents within the NTS can be eliminated as no evidence of any alteration in terminal excitability of sinus and aortic nerve afferents could be demonstrated, following conditioning stimuli to the defence area (Jordan & Spyer, 1979). In contrast our present data show an evoked hyperpolarization, and increased membrane conductance, in those NTS neurones excited by the baroreceptors following hypothalamic stimulation. This held SN (and baroreceptor)-evoked EPSPs subthreshold and in many cases shunted them. If the HDA inhibitory synaptic input is distributed, as a comparison of the duration and shape of the reversed IPSP with control would suggest (Hubbard, Llinas & Quastel, 1969), distal dendritic inhibitory

inputs might also contribute to the decreased SN-evoked EPSP duration (Sypert, Munson & Fleshman, 1980). The time course of the IPSP correlated well with the time course of inhibition noted in extracellular recordings when condition–testing paradigms were applied. We also have evidence that in some NTS neurones, particularly those excited at a longer latency by SN stimulation (Fig. 9*b*), disfacilitation as well as direct synaptic inhibition was contributing to the hypothalamic-evoked response.

The hyperpolarization evoked in NTS neurones was due to Cl^--dependent IPSPs since they could be reversed to a depolarizing wave by either Cl^- or DC current injection into the cell. The magnitude, latency and time course of these hypothalamic-evoked IPSPs were uneffected by the timing of the stimulus in the respiratory cycle. This further supports our contention (Mifflin *et al.* 1988) that there is no significant respiratory 'gating' of inputs, either afferent or descending, at this level of the NTS.

In the vast majority of cases, hypothalamic-evoked responses were unequivocally inhibitory in neurones receiving an excitatory baroreceptor input. In rare instances the IPSP was preceded by a brief EPSP, particularly when the site of stimulation was within the amygdalo-hypothalamic pathway (see also Spyer, Jordan & Wood, 1987*a*). We never observed a neurone with an exclusively excitatory input from the hypothalamus that was also excited by baroreceptor stimulation. There were, however, neurones that were excited by SN stimulation and received an excitatory input from the hypothalamic defence area but these were never excited by intra-sinus balloon inflation, and so presumably some proportion of these received a chemoreceptor input. In contrast, in the rabbit on stimulating within the central nucleus of the amygdala, which is involved in the expression of affective behaviour, a facilitatory interaction between the effects of such stimulation and baroreceptor inputs has been demonstrated (see Pascoe, Bradley & Spyer, 1987). In this species the appropriate convergence of excitatory input has been observed in neurones of the NTS (Cox *et al.* 1986). The behavioural response in the rabbit involves a marked bradycardia as part of a 'playing-dead' (Applegate, Kapp, Underwood & McNall, 1983) response unlike the defence response of the cat (Timms, 1981). It may well be that the two functional responses, and appropriate pathways, are part of the repertoire of response to stressful situations in the two animals, and merely the balance between them is different in each species. Accordingly the EPSP preceding the IPSP may reflect activation of this second system in the cat.

Observations on the different patterns of response evoked in NTS neurones on hypothalamic, SN, AN and baroreceptor activation, may provide some useful information with regard to the synaptic interconnections that exist within the NTS and are responsible for processing the baroreceptor input. We have evidence both in this and the preceding report (Mifflin *et al.* 1988) of populations of NTS neurones exhibiting latencies compatible with both mono- and polysynaptic inputs from the SN (Fig. 11, neurones B–E). We have also identified neurones that are inhibited on SN stimulation (Fig. 11, cell A), including some that have a preceding EPSP (Mifflin *et al.* 1988). The IPSP is generated polysynaptically and many of the neurones are also excited on hypothalamic stimulation, particularly in the case of those shown also to be inhibited by baroreceptor stimulation. The hypothalamically evoked

excitation may result from both direct excitation but also through disinhibition as a result of hypothalamic-evoked inhibition of antecedent neurones. The predominant effect of hypothalamic stimulation is to inhibit neurones activated by baroreceptor inputs (Fig. 11, cells B and C) and it is possible that neurones of the type indicated as A represent a local interneurone mediating this hypothalamic inhibition. In

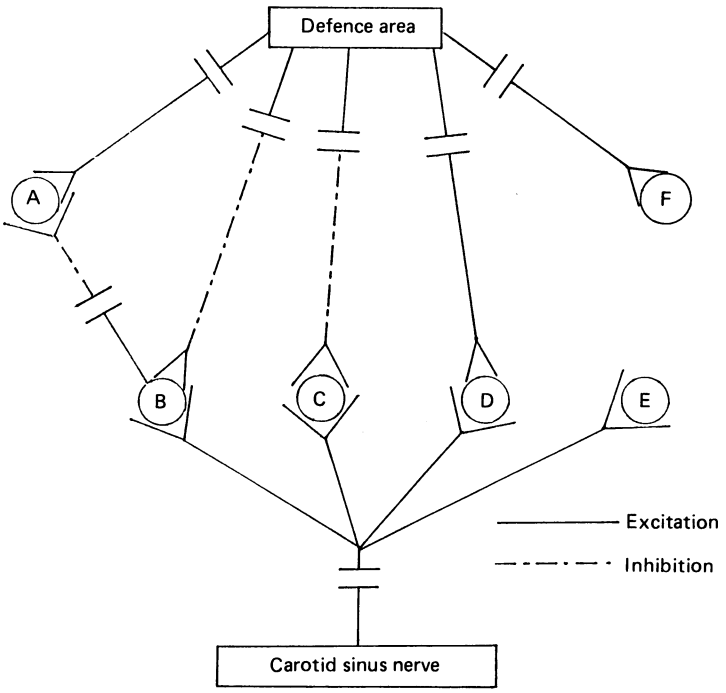

Fig. 11. Postulated interconnections between HDA and NTS. Breaks in lines indicate indeterminate number of synapses involved. SN afferent inputs excite cells B–E in NTS. Cell E represents neurones with no HDA input and cell D neurones excited by both SN and HDA stimulation. Cell C represents the majority of the population, cells excited by SN stimulation and inhibited by HDA stimulation. Cell A represents neurones inhibited by SN stimulation. Whether or not HDA stimulation excited cell A, HDA stimulation disinhibited cells in class A, presumably by inhibition of antecedent cells in class B which were excited by SN stimulation and projected to cell A. As some cells in class A were inhibited by activation of the carotid sinus baroreceptors we infer that some of the cells in class B received excitatory baroreceptor inputs. All units receiving excitatory baroreceptor inputs were in classes B and C. Cell F represents neurones receiving excitatory HDA inputs with no obvious SN input.

addition, we have observed a small number of NTS neurones ($n = 4$, Fig. 11, cell F) that had no SN or baroreceptor input but were excited from the hypothalamus and they may serve as interneurones mediating the HDA-evoked inhibition. As our experimental protocol involved first identifying neurones with an SN input, this number could be a gross underestimate. As mentioned earlier, some NTS neurones were excited by both hypothalamic and SN inputs but these never received excitatory or inhibitory inputs from the baroreceptors (Fig. 11, cell D), and several neurones with SN excitatory inputs were uneffected by hypothalamic stimulation.

The pharmacology of hypothalamically evoked inhibitions in the NTS is discussed in the subsequent paper (Jordan, Mifflin & Spyer, 1988). We are currently endeavouring to label intracellularly with horseradish peroxidase the different categories of NTS neurones summarized in Fig. 11 to determine their interconnections (Mifflin, Spyer & Withington-Wray, 1987*b*). It is attractive to suggest that the neural circuitry in the NTS offers a point at which both peripheral and central inputs involved in cardiovascular homeostasis can be integrated. With regard to central inputs the 'defence-alerting' system has often been considered to be involved in general arousal, and perhaps the sleep–wakefulness cycle, so that variations in the activity of descending inputs may well affect the efficacy of the baroreceptor control of both vagal and sympathetic outflows. In this context previous observations of a direct hypothalamic inhibitory control of the vagal outflow during the defence reaction must thus be supplemented by a disfacilitation component as a result of actions within the NTS.

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