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Pre-sympathetic neurones in the rostral ventrolateral medulla of the rat: electrophysiology, morphology and relationship to adjacent neuronal groups

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Abstract. The activity of preganglionic sympathetic neurones largely depends on synaptic excitation from antecendent reticulospinal neurones located in the rostral ventrolateral medulla (RVLM). Our study, conducted in anaesthetized rats, showed that all RVLM pre-sympathetic neurones display a substantial synaptic noise and their action potentials are usually preceded by fast EPSPs. No evidence was found for presence of gradual depolarizations (autodepolarizations) between individual spikes. Therefore our results are consistent with the "network" hypothesis for the generation of sympathetic vasomotor tone. Axons of some pre-sympathetic neurones intracellularly labelled with Neurobiotin or Lucifer Yellow had collaterals arborizing in several medullary regions. Thus these neurones have synaptic inputs not only to preganglionic sympathetic neurones, but also to other, yet unidentified cells in the brainstem. Finally, our results show that anatomically adjacent RVLM pre-sympathetic and Bötzinger respiratory neurones from two functionally distinct neuronal subpopulations, and that some pre-sympathetic neurones have an adrenergic phenotype.

Review

Key words: cardiovascular control, brainstem, intracellular recording, immunocytochemistry, *in vivo*

INTRODUCTION

There is now considerable evidence that the rostral and ventrolateral part of the medulla oblongata (the rostral ventrolateral medulla, RVLM) contains neurones that play a critical role in maintaining the activity of vasomotor preganglionic sympathetic neurones, and in evoking changes of arterial blood pressure by a variety of cardiovascular reflexes (for review see Guyenet 1990, Dampney 1994, Reis et al. 1989, 1994). The main arguments for the involvement of this brainstem region in the tonic and phasic control of the cardiovascular system are:

1. Bilateral lesioning or chemical inactivation of the RVLM in anaesthetised animals produce large falls in blood pressure;
2. Electrical or chemical stimulation of this region increases the activity of the sympathetic system and evokes large pressor responses;
3. Some RVLM neurones project to thoraco-lumbar segments of the spinal cord where they terminate on preganglionic sympathetic neurones, and to other parts of the CNS known to be involved in regulation of blood pressure;
4. The RVLM mediates and integrates most, if not all, cardiovascular reflexes/responses evoked from peripheral receptors or central sites;
5. RVLM neurones mediate the action of some centrally acting hypotensive drugs;
6. Studies using extracellular recording techniques reveal the presence in this area of tonically active, barosensitive neurones which can be antidromically excited from the thoracic spinal cord.

However, in spite of a substantial body of research, many fundamental questions regarding the identification and function of these neurones are unanswered or remain controversial. Our laboratory is particularly interested in examining synaptic inputs to the RVLM cardiovascular (pre-sympathetic) neurones, their morphology and membrane properties. In addition, we investigate the degree of anatomical and functional overlap between these neurones and other neuronal groups found in the same medullary region, including the catecholaminergic C1 neurones, respiratory neurones of the Bötzinger complex, and respiratory motorneurones in the rostral part of the nucleus ambiguus. All studies are conducted *in vivo* (in anaesthetized rats) as *in vitro* brain slices do not allow the use of the main criterion for the identification of RVLM pre-sympathetic neurones, which is the inhibition of the ongoing activity following stimulation of the arterial baroreceptors or baroreceptor afferents. Identification of baroreceptor input is crucial, as the RVLM region is functionally, as well as anatomically and chemically, heterogeneous. The results presented here summarise our recent findings on the structure and function of RVLM pre-sympathetic neurones, and their relationship to several adjacent neuronal groups (Kanjhan et al. 1995, Lipski et al. 1995a,b,c, Lipski et al. 1996).

METHODS

Experiments were conducted on pentobarbital anaesthetized (Nembutal, 80 mg/kg i.p., followed by 4-6 mg/h, i.v.), artificially ventilated, adult male Wistar rats. The end-tidal CO_2 was carefully controlled and maintained between 4 and 5%. The surgical, electrophysiological, anatomical and immunocytochemical techniques used in these experiments were described in previous publications from our laboratory (Pilowsky et al. 1990, Bryant et al. 1993, Kruszewska et al. 1994, Kanjhan et al. 1995, Lipski et al. 1995b,c, Lipski et al. 1996). In brief, after standard cannulations, dissection of several nerves (see below) and placing the animals in a stereotaxic frame, the surfaces of the medulla oblongata and spinal cord (C1 to T2) were exposed. Stimulating electrodes were placed on one or both aortic depressor nerves (ADNs), the mandibulalar branch of the facial nerve, in the lateral funiculi at the T2 segment, in the nucleus ambiguus (at obex level) and in the oesophagus. Recordings were made from the phrenic nerve and from RVLM neurones using, respectively, standard bipolar electrodes and glass microelectrodes. Low impedance microelectrodes (filled with 2M NaCl or 5% pontamine sky blue in 0.5M sodium acetate) were used for extracellular recording of unit activity or anti-

dromic field potentials in the facial nucleus or the compact formation of the nucleus ambiguus. Intracellular recordings were made with high-impedance microelectrodes usually containing 1 or 1.5% Neurobiotin or 3 (or 5)% Lucifer Yellow. Microelectrode tracking was made in a region extending 200 μm rostral to 800 μm caudal from the caudal border of the facial nucleus (as identified by recording antidromic fields from this nucleus), and ventral or ventromedial to the compact formation of nucleus ambiguus (as identified by antidromic field potentials evoked by stimulation of the oesophageal wall).

Animals in which extracellular or intracellular labelling was made, were perfused transcardially with a fixative. Fifty or 35 μm sections of medulla oblongata, caudal pons and rostral part of the first spinal segment were cut in the transverse or parasagittal plane using a Vibratome. Neurones injected with the Lucifer Yellow were directly identified with a fluorescence microscope. The sections containing such neurones were incubated with anti-tyrosine hydroxylase (TH) antibody, followed by incubation with a biotinylated secondary antibody and Streptavidin-Texas red. They were then analysed with the fluorescence microscope and confocal microscope (Leica TCS 4D system). Subsequently, they were incubated with an antibody to Lucifer Yellow, followed by an immunoperoxidase protocol. This protocol, developed in our laboratory (Pilowsky et al. 1991), leads to permanent visualisation of Lucifer Yellow labelled cell bodies and their processes for standard camera-lucida reconstructions. Sections containing Neurobiotin-injected cells were first incubated with ExtrAvidin-FITC conjugate. They were viewed under a fluorescence microscope and then incubated with a primary antibody to tyrosine hydroxylase and a secondary antibody conjugated with Texas Red. Following analysis with a fluorescence or a confocal microscope, permanently stained neurones ("hard copies") were obtained by incubating the sections in DAPA-biotin-HRP, followed by a peroxidase protocol (Bryant et al. 1993).

RESULTS AND DISCUSSION

Extracellular recordings (Kanjhan et al. 1995, Lipski et al. 1995a)

Before starting intracellular recordings, we surveyed the RVLM region with standard extracellular microelectrodes. Recording were made from 52 spontaneously active neurones (units) which were classified as pre-sympathetic on the basis of two criteria: (1) inhibition of firing after stimulation of the aortic depressor nerve (ADN) following bursts of 3 stimuli (latency, 37.3±8.3 ms after ipsilateral stimulation, and 42.7±6.0 ms after contralateral stimulation); and (2) axonal projection to the thoracic spinal cord as identified by antidromic stimulation (conduction velocity, 4.9±2.7 m/s). These neurones showed many features described in previous extracellular studies (eg. Brown and Guyenet 1985, Sun and Guyenet 1985, Morrison et al. 1988) including inhibition following pressor response after phenylephrine and cardiac rhythm at appropriate levels of blood pressure (Fig. 1A,B,C and D). However, in contrast to some studies (eg. Haselton and Guyenet 1989), they showed during normocapnia only a small degree of respiratory modulation as examined by cycle-triggered histograms (Fig. 1E). Lung hyperventilation, continued for 3 min after cessation of phrenic nerve discharge, did not significantly change the activity of most tasted neurones. These results indicate that the activity of RVLM pre-sympathetic neurones is predominantly respiratory independent.

A short lasting hypoxia (substituting nitrogen for inspired air for 10 s) resulted in variable response. About half of the tested neurones were excited, while others were not affected or weakly inhibited (not illustrated). This observation differs from the results of Sun and Reis (1993) who described hypoxic excitation of all spinally projecting RVLM barosensitive neurones.

Changes in lung volume (lung inflations) were used to assess synaptic input from lung stretch receptors. Such stimuli resulted in no significant response of most tested units (not illustrated). This result, obtained with physiological stimulation of

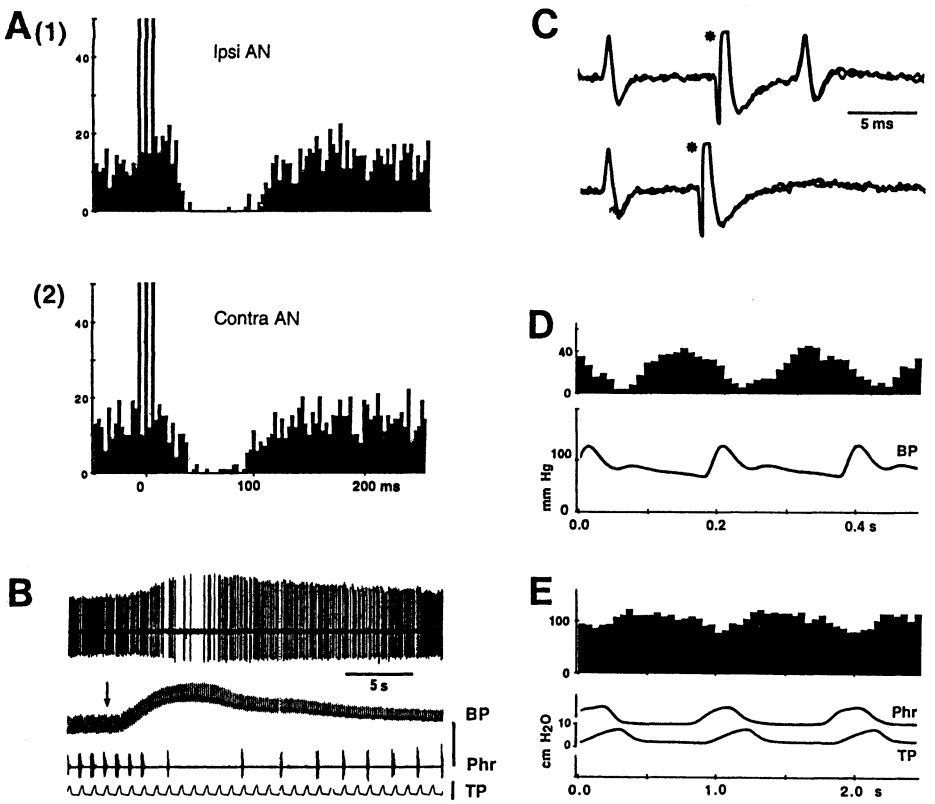

Fig 1. Identification of a pre-sympathetic neurone in the RVLM during extracellular recording. A, peri-stimulus time histograms of unit responses to stimulation of the ipsilateral (1) and contralateral (2) ADN with trains of 3 pulses. B, response of the unit to increase in blood pressure (BP, cal. bar 0-100 mmHg) evoked by i.v. administration of phenylephrine at the arrow (Phr-phrenic nerve discharge; TP-tracheal pressure, cal. bar 0-10 cm H_2O). C, antidromic response (top record) and collision (bottom record) following stimulation in the T2 spinal segment (ipsilateral side). Three superimposed sweeps in each record. Stimulus artefacts marked with asterisks. D, histogram triggered by arterial systole (BP). E, histogram triggered with integrated activity of the phrenic nerve (Phr) (end-tidal CO_2, 5%). From Kanjhan et al. (1995), with permission.

lung stretch receptors, contrasts with another study (Sun and Guyenet 1987) which described inhibition of RVLM pre-sympathetic neurones during stimulation of the cervical portion of the vagus nerve.

Extracellular deposits of pontamine sky blue confirmed that the RVLM pre-sympathetic neurones are mainly located immediately caudal to the facial nucleus, and ventromedial to the compact formation of nucleus ambiguus (Fig. 5; see also Brown and Guyenet 1985), Morrison et al. 1988).

Electrophysiological properties examined with sharp intracellular microelectrodes (Lipski et al. 1995b, Lipski et al. 1996)

The main difficulty during intracellular recording from RVLM neurones is exclusion of movement artefacts, which may produce changes of the membrane potential or firing frequency in phase with cardiac cycle, or during large changes in blood pressure (Lipski et al. 1995b). For that reason, we have assessed baroreceptor input by using stimulation of the ADN (which in the rat contains only baroreceptor afferents) with burst of pulses delivered at low frequency, which produced only minimal changes in blood pressure. Sixty-eight neurones were impaled in the RVLM region which responded with a hyperpolarizing potential (latency, 33.6±9.3 ms; amplitude 2.1±1.2 mV) following stimulation of the ADN (Figs. 2E and 3B). This inhibition was due to chloride and voltage-dependent IPSPs, as demonstrated by reversals in the polarity of these potentials during passing a continuous negative current through microelectrodes containing a low concentration of (or no) chloride ions (Fig. 2E), or following such current passed through microelectrodes containing 3M KCl. These observations provide evidence that the baroreceptor-induced inhibition of RVLM neurones is post-synaptic (ie. cannot be explained only by disfacilitation).

Some of these neurones (n=29) displayed an ongoing discharge throughout the period of recording, with the frequency of firing ranging from 5 to 43/s. The remaining 39 cells showed activity only immediately after impalement. "Spontaneously" active neurones displayed largely irregular pattern of fir-

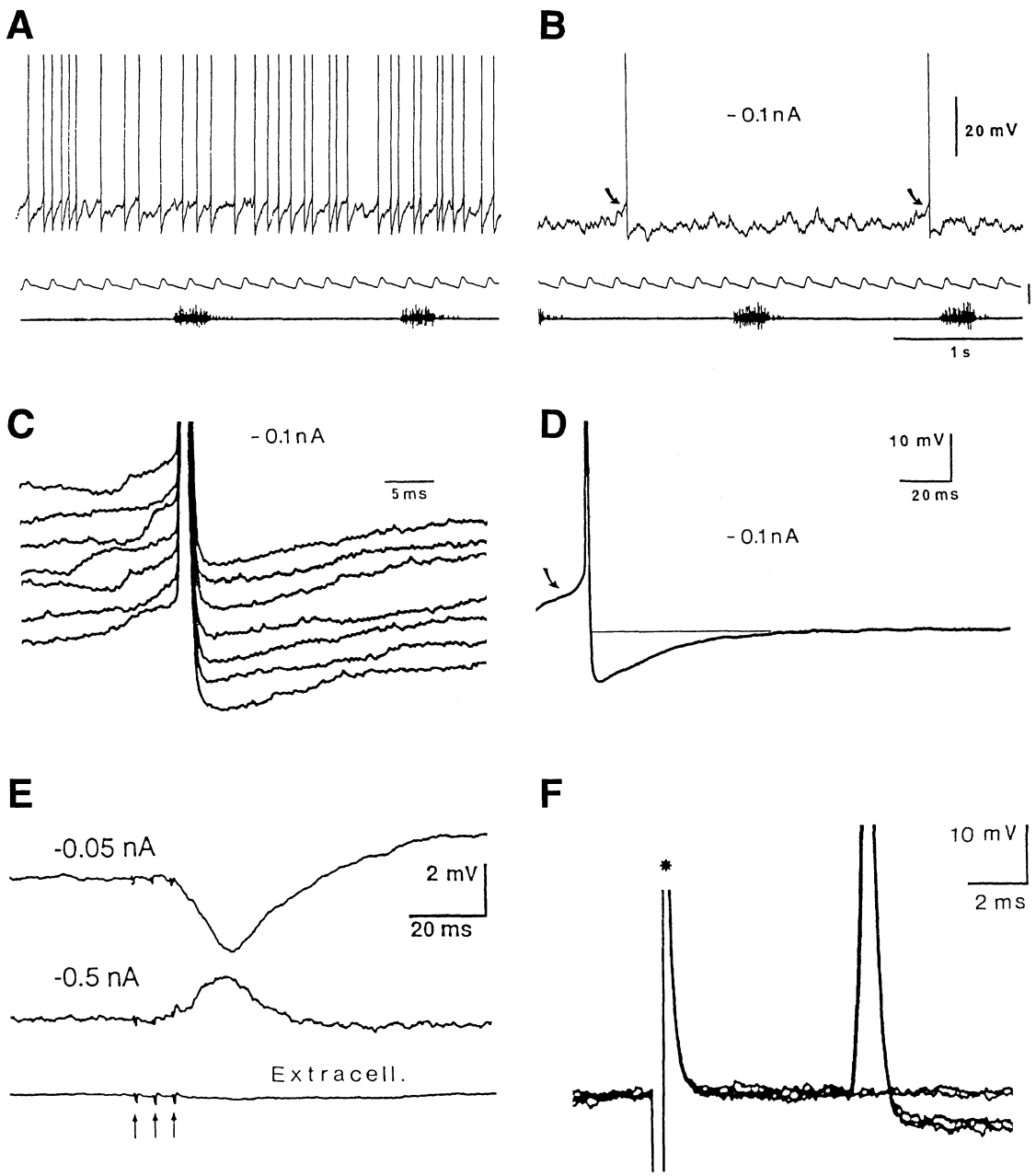

Fig. 2. Properties of an RVLM pre-sympathetic neurone revealed with an intracellular microelectrode. A, synaptic activity and firing observed when no polarizing current was used (membrane potential, -65 mV). B, synaptic activity and firing during a continuous small polarizing current. Arrows indicate large components of the synaptic noise (EPSPs) initiating individual action potentials (middle traces in A and B - arterial blood pressure, cal. bar 0-100 mmHg; bottom traces in A and B - activity of the phrenic nerve). C, EPSPs preceding 'spontaneous' action potentials, and afterhyperpolarizations following each spike shown on a faster time base, with individual sweep staggered vertically. D, time-course of afterhyperpolarizations (the area below the horizontal line) following individual action potentials (50 averaged sweeps pre-triggered with 'spontaneous' action potentials). The arrow indicates an averaged synaptic potential preceding 'spontaneous' action potentials. E, hyperpolarizing IPSP evoked by stimulation of the contralateral ADN (top trace), and its reversal during the passage of a larger polarizing current. Extracell-extracellular control. Stimulus artefacts indicated by arrows. F, antidromic action potentials evoked by near-threshold stimulation in T2 spinal segment (asterisk). Modified from Lipski et al. (1966), with permission.

ing, with no clear relationship between the level of activity (and the membrane potential) and cycles of the phrenic nerve at end-tidal CO_2 less that 5% (Figs. 2A and 3A). Action potentials were followed by short-lasting afterhyperpolarizations (amplitude, 8.6±4.5 mv; duration, 54±21 ms). Seventeen neurones could be excited antidromically after stimulation in the T2 spinal segment (Figs. 2E and 3C; conduction velocity, 4.6±1.9 m/s). In the remaining 51 neurones, spinal projection could not be tested due to the deterioration of membrane potential and spike inactivation, or due to the occurrence of short-latency (<10 ms) suprathreshold EPSPs following stimuli applied at relatively low intensity (<100 μA).

A prominent feature of all barosensitive RVLM neurones was a high level of synaptic "noise" (peak-to-peak amplitude, 8.2±3.0 mV). In spontaneously active neurones, individual action potentials were usually preceded by identifiable fast EPSPs (Fig. 2B and D). When the firing was slowed down by a small continuous hyperpolarizing current (<0.2 nA), the mean level of the membrane potential observed between individual action potentials was stable, with no evidence of depolarizing ramps (autodepolarizations) indicative of pacemaker potentials, apart from short-lasting afterhyperpolarizations which followed each action potential (Fig. 2B). Figure 2D illustrates such afterhyperpolarizations after averaging pretriggered with random spikes. Note that following the afterhyperpolarization the membrane potential is flat, as randomly occurring synaptic potentials were eliminated by averaging.

Passing a continuous hyperpolarizing current of a sufficient amplitude resulted in reversal of not only IPSPs evoked by stimulation of the ADN (see above), but also of some other tonically occurring IPSPs (not illustrated). Such IPSPs could be observed at various levels of the mean arterial blood pressure, including experiments in which blood pressure was below 80 mmHg. This observation supports the concept of an additional inhibitory input to RVLM pre-sympathetic neurones which is baroreceptor independent (eg. Dampney et al. 1988). The inhibition may originate from inhibitory interneurones found in the general area of the RVLM (eg. Millhorn et al. 1988), or from distant neurones probably located in the caudal ventrolateral medulla (eg. Cravo and Morrison 1993).

It is still not clear what are the sources of synaptic excitation of the RVLM pre-sympathetic neurones. Previous studies have identified a number of central

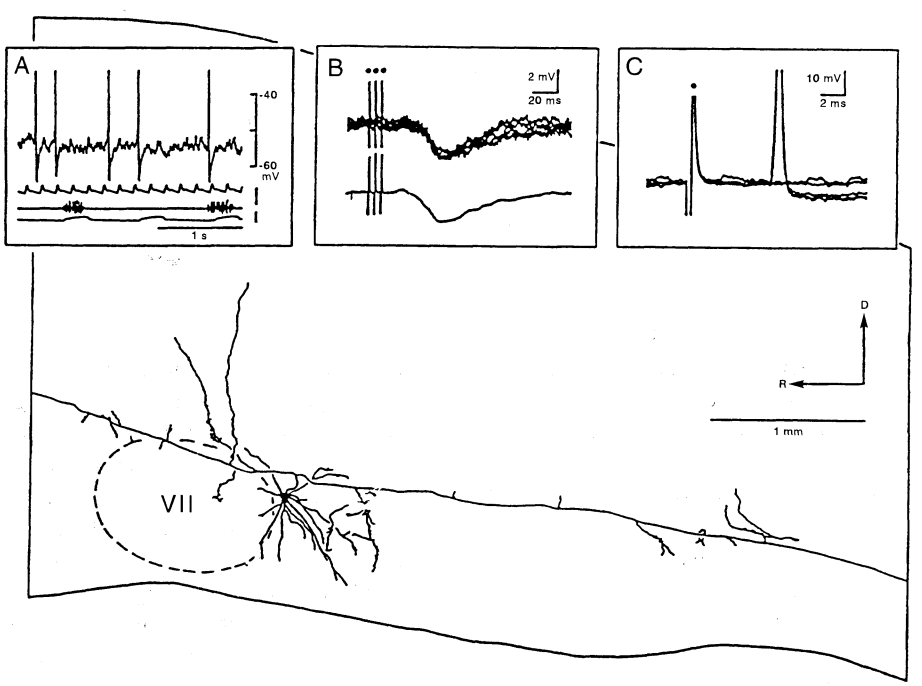

Fig. 3. Example of intracellular recording from a presympathetic neurone in the RVLM (A-C), and a camera-lucida reconstruction, in the parasagittal plane, of the same neurone filled with Neurobiotin. A, activity of the neurone in relation to blood pressure, phrenic nerve discharge, and tracheal pressure. B, IPSPs following stimulation of the ADN. C, antidromic responses following stimulation in T2 segment. Note axon collaterals originating from two main axonal branches. From Lipski et al. (1995c), with permission.

sites from which excitation of these neurones can be evoked following electrical or chemical stimulation. However, so far only two brainstem regions were identified which may provide a "tonic" excitatory drive: the lateral tegmental field in the dorsal reticular formation of the medulla oblongata (Barman and Gebber 1987), and pontine reticular formation (Hayes et al. 1994). The EPSPs responsible for eliciting a tonic outflow from pre-sympathetic neurones are likely to be mediated by excitatory aminoacids. This conclusion is not in conflict with previous studies which reported either an increase, no change, or a decrease of blood pressure or sympathetic nerve activity following a bilateral microinjection into the RVLM (or intracisternal administration) of an aminoacid antagonist kynurenic acid, blockers of the non-NMDA receptor, or cobalt (eg. Guyenet et al. 1987, Trzebski and Baradziej 1992, Abrahams et al. 1994). The variability of the response may be due to the fact that these drugs (with the exception of cobalt which can block transmission in all synapses) not only block excitatory receptors on pre-sympathetic neurones, but also on other interneurones, including inhibitory interneurones. If these interneurones are also tonically excited by synapses utilizing an excitatory aminoacid, then such chemical deafferentation would not only lead to disfacilitation of pre-sympathetic neurones, but also to their disinhibition. The overall effect will depend on the balance between direct excitatory and inhibitory inputs to pre-sympathetic neurones, and to antecedent neurones. It is likely that this balance is affected by experimental conditions, for example the kind of anaesthesia.

As indicated above, under our experimental conditions we found no evidence that the activity of RVLM pre-sympathetic neurones depends on intrinsic pacemaker properties, and therefore our results are consistent with the "network" hypothesis of generation of sympathetic vasomotor tone, as suggested by Barman and Gebber (1987). The "pacemaker" hypothesis of sympathetic tone generation was originally proposed by Sun, Guyenet and their colleagues (eg. Sun et al. 1988) mainly on the basis of the intracellular studies conducted in medullary slices, and extracellular recordings *in vivo* in the presence of kynurenic acid. We propose that the regular pattern of activity observed in tissue slices (and *in vivo* after kynurenate) may be the result of deafferentation, and that the pacemaker-like properties observed in such preparations play little, if any, role in controlling the activity of pre-sympathetic neurones *in vivo*. The key element of our hypothesis is an assumption that the *in vitro* isolation or intracisternal administration of kynurenate are associated with reduction or elimination of not only EPSPs, but also of inhibitory inputs from both local and distant inhibitory interneurones. If the inhibitory inputs normally prevail, then *in vitro* isolation or *in vivo* administration of kynurenic acid could lead to disinhibition, threshold depolarization and firing. Interestingly, the presence of pacemaker-like activity in cells isolated *in vitro*, but its lack in the whole animal has previously been reported also in other neuronal systems. For example, Dembowsky (1995) observed the presence of pacemaker potentials in preganglionic sympathetic neurones *in vitro* but not *in vivo*. Similarly, in a classic invertebrate pacemaker, R15 neurone in the abdominal ganglion of Aplysia, oscillations of the membrane potentials do not occur if recordings are made in the intact animal (Alevizos et al. 1991).

Morphology (Lipski et al. 1995c)

Thirty neurones displaying IPSPs following stimulation of the ADN were intracellularly labelled with a fluorescent dye Lucifer Yellow, or a biotin-like tracer, Neurobiotin. Some of these neurones could be excited antidromically from T2 segment of the spinal cord, with conduction velocities of spinal axons ranging from 1.9 to 7.2 m/s. The filled somas were found immediately caudal to the facial nucleus and ventral or ventromedial to compact formation of the nucleus ambiguus, the location described in previous extracellular studies. The dendritic trees were moderately extensive, with the longest dendrite, identified in the transverse plane, extending 780 μm from the cell body. Some dendrites reached the ventral medullary surface. Stem

axons usually projected dorsomedially and then made a sharp rostral and/or caudal turn. The caudally projecting axon could, in some cases, be followed to the first cervical segment of the spinal cord. Interestingly, seven of the 14 reconstructed cells issued fine axon collaterals which were identified, on the ipsilateral side, in two areas: in the RVLM (or immediately dorsomedial to that region) and within the dorsal vagal complex. Figure 3 illustrates an example of a camera-lucida reconstruction of a Neurobiotin-filled neurone.

Although several previous studies described the morphology of putative pre-sympathetic RVLM neurones *in vitro* (eg. Sun et al. 1991), our study was the first which described the morphological properties of cells positively identified as barosensitive. In addition, we could trace axons and axon collaterals for large distances from the cell bodies, which is not possible in tissue slices. Our results show that cardiovascular RVLM neurones are heterogenous also with respect to their morphology, and the differences include the shape and size of cell bodies, presence or absence of spinal projection, rostral axonal projection within the brainstem (presumably to the locus coeruleus, hypothalamus and dorsolateral pons; eg. Tucker et al. 1987, Pierbone et al. 1988), and the presence of axon collaterals within the medulla oblongata. These observations are consistant with the view that the function of at least some RVLM pre-sympathetic neurones extends beyond controlling the activity of preganglionic sympathetic neurones (Haselton and Guyenet 1990).

Relationship to C1 adrenergic neurones (Lipski et al. 1995c)

Previous studies indicate that RVLM pre-sympathetic neurones use glutamate as the main neurotransmitter (eg. Morrison and Reis 1991, Chalmers et al. 1992). A subset of RVLM neurones with a catecholaminergic (adrenergic) phenotype, known as the C1 cell system, may also release adrenaline as a co-transmitter. Although these adrenergic neurones have been implicated in the control of the cardiovascular system (eg. Reis et al. 1989), their involvement in excitation of preganglionic sympathetic neurones remains controversial. In addition, there has been no direct evidence that these adrenergic neurones have an inhibitory baroreceptor input.

Twenty-seven barosensitive RVLM neurones (including bulbospinal, and non-spinally projecting neurones) were intracellularly labelled with Lucifer Yellow or Neurobiotin, and examined under a fluorescence or confocal microscope for evidence of immunoreactivity for tyrosine hydroxylase (TH). Seven of these cells (26%) were double-labelled (ie. were TH-immunoreactive) and were classified as C1 adrenergic neurones. Positive staining for TH was considered a marker for adrenergic neurones, as previous studies reported that in the rat, virtually all RVLM neurones immunoreactive for TH also stain for phenylethanolamine N-methyltransferase (PNMT). An example of confocal microscope image of a neurone filled with Lucifer Yellow which was TH-immunoreactive is shown in Fig. 4.

TH-immunoreactivity was not detected in the 20 remaining intracellularly labelled neurones. Most of these neurones were in close proximity to other immunoreactive cell bodies. As the histological sections were relatively thick, one factor which could explain the lack of double-labelling in this group of cells is incomplete penetration of the immunoreagents into the sections. This factor was excluded following the analysis of confocal z-scans, which revealed the presence of TH-immunoreactive cell bodies and processes not only near the surface, but also in the middle of the sections.

A relationship was found between the presence or absence of TH-immunoreactivity and the amplitude of ADN-evoked IPSPs. In the TH-negative cells, the amplitude was 1.2±0.7 mV, and in the TH-positive neurones 2.9±1.4 mV ($P<0.01$). The bigger amplitude of IPSPs in TH-immunoreactive neurones could be due to their higher input resistance, larger synaptic currents generated by antecedent inhibitory interneurones (presumably located in the caudal ventrolateral medulla; Jeske et al. 1993), or simply to greater damage and depolarization of adrenergic neurones resulting from impalements. The contribution of these factors awaits future investigation.

The proportion of double-labelled cells (26%) was lower than estimates based on previous anatomical studies examining spinally projecting C1 cells, which showed that 45-72% of the RVLM neurones that were retrogradelly labelled from the thoracic segments of the spinal cord also stained for TH or PNMT (eg. Tucker et al. 1987, Ruggiero et al 1994). However, our results show that a proportion of spinally projecting neurones in this region are non-adrenergic (see also Sun et al. 1988). Clearly, some spinally projecting neurones are not barosensitive (Kanjhan et al. 1995). In addition, at least some barosensitive neurones which do not project to the spinal cord belong to the non-adrenergic group, therefore, would not be included in estimates based on anatomy and immunocytochemistry. These factors may have contributed to an overestimation of the proportion of C1 neurones involved in the control of vasomotor preganglionic sympathetic neurones and in mediating the baroreceptor reflex.

Relationship to respiratory neurones of the Bötzinger complex (Kanjhan et al. 1995)

The Bötzinger complex (Lipski and Merrill 1980) consists of expiratory neurones (ie. neurones active in the expiratory phase of the central respiratory cycle) which are propriobulbar or project to cervical segments of the spinal cord (eg. Lipski and Merrill 1980, Otake et al. 1987, Jiang and Lipski 1990, Bryant et al. 1993). It is still not clear whether this group of neurones is distinct from pre-sympathetic neurones with respect to electrophysiological properties and anatomical location. The aim of this part of our study was to investigate the degree of functional and anatomical overlap between these two neuronal subpopulations in the RVLM. Extracellular recordings were made with dye-filled microelectrodes. Units were classified as Bötzinger expiratory neurones on this basis of two criteria: (1) distinct ("on-off") respiratory pattern of activity in normocapnia, with action potentials present during central expiration; and (2) axonal projection to caudal parts of the nucleus ambiguus (at the obex level) on the ipsi- or contralateral side, to distinguish from respiratory motoneurones located in the semicompact formation of the nucleus ambiguus (Bryant et al. 1993). Pre-sympathetic neurones were identified as described above. The tests applied included stimulation of the ADN, short-lasting activation of peripheral chemoreceptors, activation of lung stretch receptors, changes in central respiratory

Fig 4. Scans made on the confocal microscope of the pre-sympathetic neurone double-labelled for Lucifer Yellow (left) and tyrosine hydroxylase (right). Tyrosine hydroxylase was detected with an antibody conjugated to Texas Red. Vertical bars show grey-scale for each image.

drive with hyper- or hypoventilation, nociceptive stimulation and antidromic stimulation from the T2 segment of the spinal cord or medulla oblongata at obex level.

The two groups of neurones (pre-sympathetic and Bötzinger) showed not only different patterns of spontaneous activity, but also generally different responses to these stimuli. The recording positions showed some overlap, but the majority of Bötzinger neurones were dorsolateral to pre-sympathetic neurones (Fig. 5). There was a large overlap between the location of pre-sympathetic neurones and the lateral part of the C1 adrenergic group, but there was only a small overlap between these adrenergic neurones and Bötzinger neurones (see also Pilowsky et al. 1990).

These results indicate that the anatomically adjacent pre-sympathetic and Bötzinger neurones form two functionally distinct neuronal subpopulations. Their overall features are consistent with the concept that pre-sympathetic neurones are primarily engaged in control of the cardiovascular system, while Bötzinger neurones play a role in generation of respiratory motor output. Therefore, these two neuronal groups are unlikely to be part of the "common cardiorespiratory network" as suggested by others (Koepchen et al. 1981, Richter et al. 1991). However, this conclusion does not exclude the existance of a network involving other groups of cardiovascular and respiratory neurones not included in the present investigation.

CONCLUSIONS

Our electrophysiological data extends the information on the electrophysiological properties of RVLM pre-sympathetic neurones. In particular, we have demonstrated that the activity of these neurones during normocapnia is predominantly respiratory independent. Their baroreceptor-induced inhibition is post-synaptic and results from chloride and voltage-dependent IPSPs. Under our experimental conditions, all barosensitive RVLM neurones displayed large synaptic activity and individual action potentials were preceded by identifiable fast EPSPs. No evidence was found for the presence of gradual depolarizations (autodepolarizations) between individual action potentials, and therefore *in vivo* the activity of these neurones does not depend on intrinsic pacemaker properties. We suggest that the regular firing and other pacemaker-

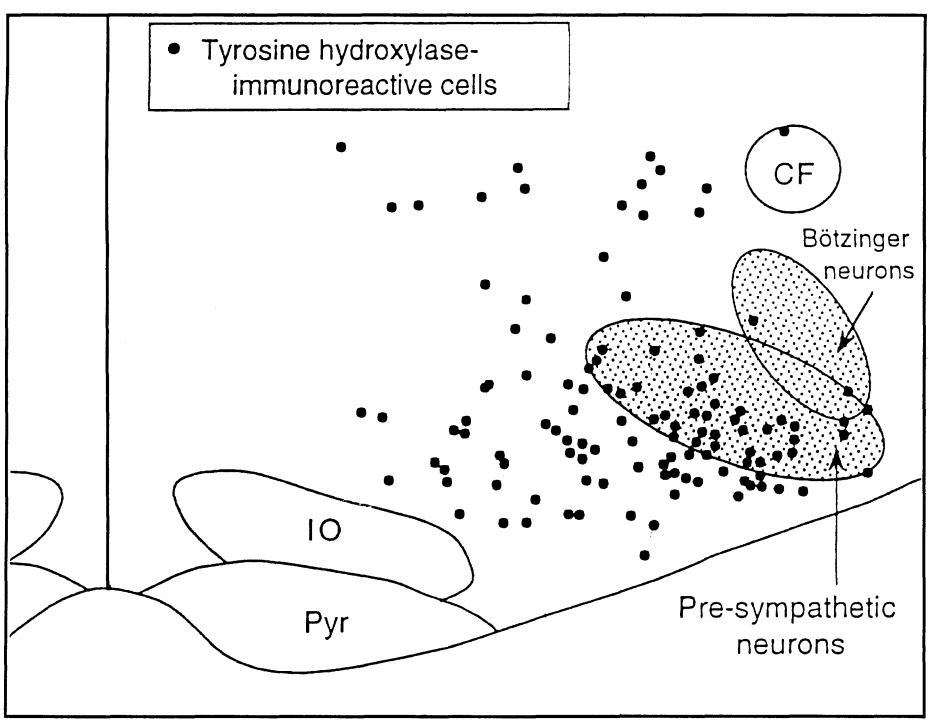

Fig. 5. Location of tyrosine hydroxylase-immunoreactive cells and projected areas of recording from pre-sympathetic and Bötzinger neurones. The distribution of immunoreactive cells is a composite of four 50 μm coronal sections taken from one rat between the caudal border of the facial nucleus and 450 μm caudally. CF, compact formation of nucleus ambiguus, IO, inferior olive (rostral end); Pyr, pyramidal tract. Modified from Kanjhan et al. (1995), with permission.

like properties described by others in this group of neurones may have resulted from a deafferentation associated with obtaining tissue slices, or application of antagonists of glutaminergic transmission.

The use of intracellular labels allowed us to examine the morphology of these neurones and their relationship to C1 adrenergic neurones. The presence of axon collaterals and bouton-like varicosities in several brainstem regions suggest that the function of at least some of these neurones extends beyond their role in exciting preganglionic sympathetic neurones. However, the exact synaptic targets for these collaterals remain to be identified. Our experiments also provided a direct demonstration that some barosensitive RVLM neurones have an adrenergic phenotype. However, the proportion of double-labelled cells was lower than in previous estimates and, therefore, our results support the view that the non-catecholaminergic RVLM neurones also play an important role in vasomotor control. Finally, our study provides additional evidence that the RVLM region is functionally heterogenous, and that the anatomically adjacent pre-sympathetic and Bötzinger expiratory neurones form two functionally distinct neuronal subpopulations.

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