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THE RHYTHMICITY OF SYMPATHETIC NERVE ACTIVITY

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Abstract—This review focuses on that most engaging feature of the sympathetic nervous system, its rhythmicity. In particular examining the nature of sympathetic nerve activity (SNA), its characteristics, the frequencies of these rhythms and possible mechanisms responsible for their generation. Sympathetic activity can be thought of as a complex output of the central nervous system providing subtle control over end organ function. This control is exerted in a number of frequency bands including rhythms related to the cardiac and respiratory cycles, 10 Hz, and between 0.2 and 0.4 Hz. The generation and control over the occurrence of each of these rhythms is likely to be quite separate. Although afferent feedback from sources such as baroreceptors can explain some of the rhythmical properties in each case there is good evidence for inherent generation of aspects of these rhythms. A variety of brainstem cell groups are thought to be involved in their generation with the rostral ventrolateral medulla, although unlikely to be solely responsible for tone generation, an important regulator of overall activity. SNA also varies in the number of nerves recruited to fire in each synchronized discharge. Little is known about this control other than it appears to be quite separate from the control over the timing of discharges. Spinal cord mechanisms are possibly involved. SNA frequencies above 0.7 Hz do not appear to directly induce oscillations in innervated vasculature, however, are likely to contribute to setting the level of vasoconstrictive tone. Slower frequencies appear to directly cause oscillations in blood flow.

CONTENTS

1. INTRODUCTION

Sympathetic nervous system activity provides one of the fundamental aspects in the control of arterial pressure. By rapidly regulating the level of activity it alters the degree of vasoconstriction in the blood vessels of many key organs around the body. This in turn increases or decreases blood flow through organs, affecting the function of these organs and arterial pressure. In contrast to the activity present in motor nerves, sympathetic nerves are continu-

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ously active meaning all innervated blood vessels remain under some degree of continuous constriction. Since its first description in the 1930s (Adrian *et al*., 1932; Bronk *et al*., 1936) sympathetic nerve activity (SNA) has engendered itself to researchers in two camps; neurophysiologists have seen its inherent properties as an opportunity to understand how areas of the central nervous system may be wired up to generate and control such activity (Gebber, 1976; Kocsis *et al*., 1993; McAllen and May, 1996), while cardiovascular physiologists saw its regulation of blood flow as a direct index of circulatory control in response to different stimuli, drugs and pathological conditions (Dibona *et al*., 1996c; Head *et al*., 1997; Mark, 1990). This review focuses on that most engaging feature of the sympathetic nervous system, its rhythmicity. In particular focusing on the nature of SNA, its characteristics, the frequencies of these rhythms and responses to different stimuli. It shall not deal in detail with the anatomical and functional organization of pathways within the brain involved in regulating overall SNA unless it is pertinent to understanding the origin of the rhythms in SNA, as these have been dealt with already in a recent review (Dampney, 1994a).

2. OBSERVATIONS FROM EARLY RECORDINGS

Evidence that sympathetic nerves are tonically active was established from the 1850s with the observation that section or electrical stimulation of the cervical sympathetic nerve led to changes in blood flow in the rabbit ear (Bernard, 1851). However, it was not until the 1930s that Adrian *et al*. published the first description on the nature of actual sympathetic discharges (Adrian *et al*., 1932). An original neurogram from a multifibre nerve recording of cardiac SNA in the anesthetized cat from their work (Bronk *et al*., 1936) is shown in Fig. 1 and illustrates SNA's two most obvious features; that discharges occur in a synchronized fashion, with many of the nerves in the bundle being active at approximately the same time, secondly that they occur with each cardiac cycle in a highly rhythmical fashion. They also noted that by no means was the overall activity level constant as it was increased by asphyxia or a small fall in blood pressure. This was the first direct evidence supporting Hunt's assertion in 1899 (Hunt, 1899) that the heart is under the continual influence of sympathetic impulses. These early papers answered a number of simple questions on the nature of the multifibre discharges, such as whether the activity reflected that of single fibres or groups working more or less synchronously, and if it occurred in both pre- and postganglionic fibres. They pointed out the considerable variation in the amplitude of discharges which did not vary much in 'contour' or duration. They also showed that the synchronized activation of postganglionic nerves was not a function of the ganglia as it could be observed in preganglionic nerves and that activity was bilaterally synchronous. That is, that activity in right and left cardiac nerves was the same.

The origin of the rhythmical discharges was considered in the 1930s to be a simple consequence of phasic input from arterial baroreceptors, which had recently been shown to display pulsatile activity (Bronk and Stella, 1932). This proposal had the effect of diminishing the role of the central nervous system to that of a simple relay station and may go some way to explaining the lack of further interest in recording SNA until the late 1960s. Green and Heffron (1967) then re-examined the question of the origin of SNA after noting a rapid sympathetic rhythm (at *ca* 10 Hz) under certain conditions (mainly reduced baroreceptor afferent traffic) that was far faster than the cardiac rhythm. This immediately indicated that the origin of bursts of SNA could not simply be a product of regular input from baroreceptors. Their suggestion that the fast rhythm did not have a cardiac or ganglionic origin, but was of brain stem origin had the net effect of transforming the area from one of solely circulatory control to one that neurophysiologists could use for the study of the central nervous system.

3. WHAT IS A DISCHARGE OF SYMPATHETIC NERVE ACTIVITY?

Postganglionic sympathetic nerves are composed of thousands of unmyelinated fibres (DiBona *et al*., 1996b), whose individual contributions to the recorded signal are exceedingly small. But fortunately, their ongoing activity can be measured from whole nerve recordings because large numbers of fibres fire action potentials at almost the same time, to give discharges of summed spikes. Although there is no way of knowing the actual number of fibres

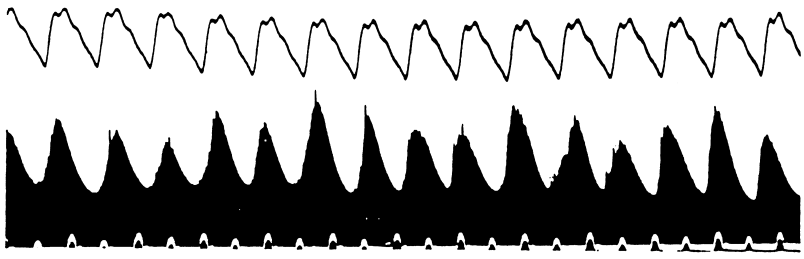

Fig. 1. Original neurogram from a multifibre nerve recording of cardiac SNA (bottom panel) in the anesthetized cat (top panel is arterial pressure, both signals were uncalibrated). It should be noted that this figure over represents the rhythmicity of SNA, suggesting it is extremely regular. In general its activity is much less rhythmical as can be seen in later figures. After (Bronk *et al*., 1936).

activated in each discharge it is possible to make an estimation of the relative number of fibres currently active by using stimuli which recruit almost all the nerves. In the conscious rabbit this can be performed by puffing a small amount of smoke in the face of the animal. This nasopharyngeal stimulation evokes the largest known recruitment of nerves (Dorward *et al.*, 1985). Using this stimuli, where mean renal SNA can increase up to 500%, it has been estimated that resting nerve activity may only comprise the activation of 10–20% of the nerves in the bundle (Malpas *et al.*, 1996).

Although it is possible to perform single unit recordings from postganglionic nerve fibres (Boczek-Funcke *et al.*, 1992a; Dorward *et al.*, 1987; Kollai and Koizumi, 1980), the favoured approach is a multiunit recording. This is obviously a much easier experimental preparation, allowing recordings in conscious animals and giving overall responses similar to that recorded from a single unit (Häbler *et al.*, 1993). The main disadvantage is that due to the physical nature of contact between the recording electrode and the nerve it is not generally advisable to compare the voltages obtained between animals, although some researchers have attempted this (Morgan *et al.*, 1995). There are, however, several important points that can only be shown from single unit recordings; firstly, while multifibre discharges can occur at quite fast rates (up to 10 Hz, see a later section) the frequency of firing in the single unit is much lower. Average rates in chloralose-urethane or alfathesin anesthetized rabbits have been recorded between 2 and 2.5 spikes sec^{-1} for renal nerves (Dorward *et al.*, 1987) and *ca* 1.2 spikes sec^{-1} for splenic nerves in the cat (Meckler and Weaver, 1988). How this relatively slow rate of firing summates to form high frequency multiunit discharges is represented in Fig. 2. This slow firing rate means that the rhythmical properties of the discharges are not seen unless their activity is averaged over time against a reference such as the cardiac cycle or respiration (Dorward *et al.*, 1987). Single unit recordings also show the minimal firing interval for postganglionic neurons is between 90–100 msec (Ootsuka *et al.*, 1995). This indicates it is unlikely that multifibre discharges represent high frequency impulses from a single neuron but rather the summation of impulses from multiple fibres that fire synchronously. The other important feature observable in single unit recordings is the relationship of the individual action potentials to the cardiac cycle, that is although the average discharge rate is low, when the nerves do fire, they do so at approximately the same time in the cardiac cycle (Fig. 3). Recording of single unit activity also gives information on the population properties of the multifibre preparation. For example, whether nerve fibres function as a homogeneous population or contain subpopulations with different responses to stimuli. In the case of the renal nerves the active portion of the population seem to be relatively homogeneous with uniform firing properties, conduction velocities and responses baroreceptor and chemoreceptor activation (Dorward *et al.*, 1987). However, a subpopulation of nerves, normally silent, can be activated under thermal stimuli (DiBona *et al.*, 1996b). The problem with such knowledge is that it is difficult to ascribe some functional relevance to these different properties, as it is impossible to identify the termination of the nerves being recorded from in the kidney. Jänig has reviewed the different types of neuronal discharge patterns based on functional properties of the vasoconstrictive neurons supplying skeletal muscle of the cat hindlimb, and hairy and hairless skin (Jänig, 1988). There are quite clear differences in the activity depending on its terminus. Activity to the muscle is activated by inhibition of arterial baroreceptors, or stimulation of chemoreceptors or nociceptors. In comparison the cutaneous vasoconstrictor neurons are only weakly affected by arterial baroreceptor activation but are activated by most natural stimuli of afferent inputs, such as vibration and nociception (Blumberg *et al.*, 1980; Jänig and McLachlan, 1992). Such analysis has also been completed in the human for single neurons supplying the sweat glands (Macefield and Wallin, 1996) and muscles (Macefield *et al.*, 1994) (see section on human SNA).

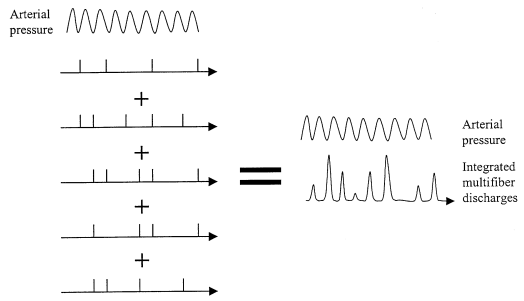

Fig. 2. Schema illustrating how the summation of activities from single neurones with low firing rates, although discharging relative to the cardiac cycle, can form synchronized bursts with a higher frequency. For clarity the multifiber bursts are integrated with a short time constant, this reflects the mass activation of single units as a burst with greater amplitude.

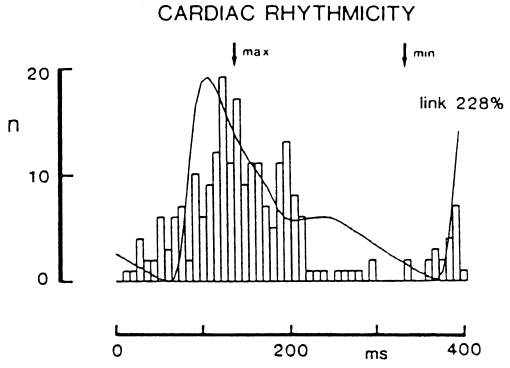

Fig. 3. Cardiac rhythmicity of single renal neuron displayed by post-R wave interval histogram, obtained from 390 cardiac cycles, at a bin width of 8 msec, *n*, number of spikes per bin. The tracing gives the average arterial pressure pulse over the sampling period and the arrows show the timing of maximum and minimum excitation. After (Dorward *et al.*, 1987).

4. SYNCHRONIZATION

The summation of individual action potentials to form bursts is generally termed synchronization of discharges. It is worthwhile to dwell on some of the mechanisms that may regulate this feature, although it is difficult to be definitive since little research has been conducted in this area. The low firing rate of individual nerves seems to preclude the same neuron being activated more than once in each multifibre discharge (Dorward *et al*., 1987; Johnson and Gilbey, 1996; Kollai and Koizumi, 1980). Rather it would seem that the activated neurons are drawn from a neuronal pool. It is unlikely that the low firing rate is due to a long refractory period for the nerves since the individual nerves can be induced to fire at quite fast rates (Dorward *et al*., 1987; Macefield *et al*., 1994).

The width of a discharge is proportional to the size of the discharge (Malpas and Ninomiya, 1992d). It is most likely that the width reflects the number of nerves recruited (see section on the amplitude of sympathetic discharges). Thus a large discharge recruiting more nerves is associated with a greater width, as measured from the integrated signal. With regard to the renal nerves this is not surprising, for although the conduction velocities are similar (DiBona *et al*., 1996b; Dorward *et al*., 1987) they will not be exactly the same, therefore any activation will produce some spread or apparent width in a multifibre discharge. It does appear that whatever mechanism coordinates the nerves to synchronously discharge this is a stable factor, as the width of discharges which have the same height are very similar (Malpas and Ninomiya, 1992d).

It should be noted that a limitation of multifibre recordings occurs if there are single nerve fibres which are tonically active, but which discharge in a non-synchronized fashion, for example not related to the cardiac cycle. These will not be observable in the recorded signal and may be counted within the noise of the signal. Such possibilities exist for nerves that may perform non-vasoconstrictive functions, for example, to regulate sodium excretion in the proximal tubule of the renal nephron (DiBona *et al*., 1996b) or having a thermoregulatory function in the case of the skin SNA (Macefield and Wallin, 1996).

Kocsis *et al*. (1993) have investigated the degree of synchronization of nerve discharges. They reasoned that analysing the dynamics of synchronization and making comparisons between different nerves to different target organs might give some insight into the differential mechanisms controlling SNA to different end organs. Using cerebral ischemia they observed a two phase response; increases in the amplitude and frequency of synchronized discharges, then a change to desynchronized activity with high frequency components starting to dominate the signal. Importantly they found that the time at which this second phase began was different between nerves to the heart, kidney and muscle. This indicates that the control over the synchronization of the bursts is not the same across all nerves and may reflect, as they propose, different central networks controlling different cardiovascular effectors.

5. WHY GENERATE SYNCHRONIZED ACTIVITY?

Unfortunately this remains an unanswered question. Indeed, what advantage does it confer to circulatory control to have coordinated bursts of nerve activity? Evidence suggests that it is not just nerves to a single organ that display bursts, but activity travelling to organs such as the heart and kidney display a high degree of coherence in the timing of their bursts (Gebber *et al*., 1994a; Kocsis, 1994). This suggests a common initiator for the synchronization of discharges although there may be separate control over the overall level of SNA. This does not, however, tell us why activity in individual nerves is coordinated to form synchronized activity. Indeed it could be argued that the uncoordinated firing of the individual neurons would give the same level of control as the synchronized discharges, providing the overall level of activity was the same. This hypothesis is in some way supported by evidence that the blood vessels do not respond to the individual discharges with vasoconstriction (Janssen *et al*., 1997). Thus, an average discharge rate between 2 and 6 Hz does not lead to a 2–6 Hz cycle of vasoconstriction and dilation in the vasculature as the time constants for responses to changes in sympathetic activity at the neuromuscular junction are in the order of 1–2 - sec (Hirst and Edwards, 1989; Somlyo and Somlyo, 1990). However, while there are no direct studies indicating the rational behind synchronized discharges for cardiovascular control, indirect evidence and theoretical studies suggest that such coordination leads to an increase in the gain of the system. That is, to have a signal where many thousands of nerve fibres are activated synchronously with a level of activation which may vary in both frequency and amplitude domains, greatly increases the number of responses that can be configured to different stimuli. Birks (1978) showed that electrical stimulation of preganglionic neurons with patterned stimulation rather than constant frequency stimulation increased the acetylcholine output of the terminals by as much as threefold. It was also shown that patterned stimuli assists the recruitment of a broader range of neurons than could be recruited by simple constant frequency stimuli (Birks *et al*., 1981). Such patterning may be an important mechanism for the modulation of synaptic transmission in sympathetic ganglia.

It could be argued that synchronization simply reflects the nature of the inputs to the circuits generating sympathetic tone and serves no real functional purpose, however, there is some evidence that the coordinated nature of the discharges may lead to a coordinated release of neurotransmitter at the neuromuscular junction (Bao, 1993; Stjarne and Stjarne, 1995). While the blood vessels may not constrict and relax with each discharge they may respond to the coordinated mass release of neurotransmitter with sustained contraction (Janssen *et al*., 1997). Andersson (1983) tested whether electrical

stimulation patterned into high frequency bursts every 10 sec was better at achieving vasoconstriction than continuous regular impulses (total number of pulses being the same). Both types of stimulation were capable of evoking maintained constriction in cat skeletal muscles, but there were no differences between the pattern of stimuli unless the burst frequency was very high. However, Hardebo (1992) took a more accurate approach by measuring the release of noradrenaline and tested whether the release would be greater with high frequency burst stimulation rather than continuous low frequency stimulation, again with the same total number of pulses. With regard to the rat caudal artery, burst stimulation at a net frequency of 6 Hz resulted in a 44% greater contractile response than using equally spaced stimuli. Furthermore, the noradrenaline release to each form of stimuli was also compared after electrical field stimulation on rat pial and caudal arteries, and rabbit ear and basilar arteries. In all vessel segments studied there was a higher release of noradrenaline when stimulation was in coordinated bursts, similar to that occurring in tonic SNA, rather than continuous train stimuli.

It also appears that different frequencies of SNA can evoke different neurotransmitter responses. With electrical stimulation at low frequencies the neurotransmission was shown to be highly calcium dependent, but not during higher frequency stimulation (Sjöblom-Widfeldt and Nilsson, 1989). Additionally, high frequency stimulation of the nerves in small mesenteric arteries of the rat mainly evoked the release of noradrenaline, while slower frequency stimulation involved an undetermined non-adrenergic transmitter (Sjöblom-Widfeldt and Nilsson, 1990). Similar responses were observed for the pig spleen, where the release of neuropeptide Y (NPY) was enhanced by electrical stimulation at frequencies < 2 Hz (Pernow *et al.*, 1989). Higher frequencies enhanced both NPY and noradrenaline release. The central ear artery of the rabbit also shows different responses to slow and fast frequency stimuli, low frequencies favouring a purinergic response and faster frequencies the noradrenaline component (Kennedy *et al.*, 1986). Such findings suggest that the different frequencies of SNA do not simply mean greater or lesser vasoconstriction in the innervated vasculature but may reflect quite different phenomena with different latencies and refractory periods, and thus different functional responses as a result of different neurotransmitters involved.

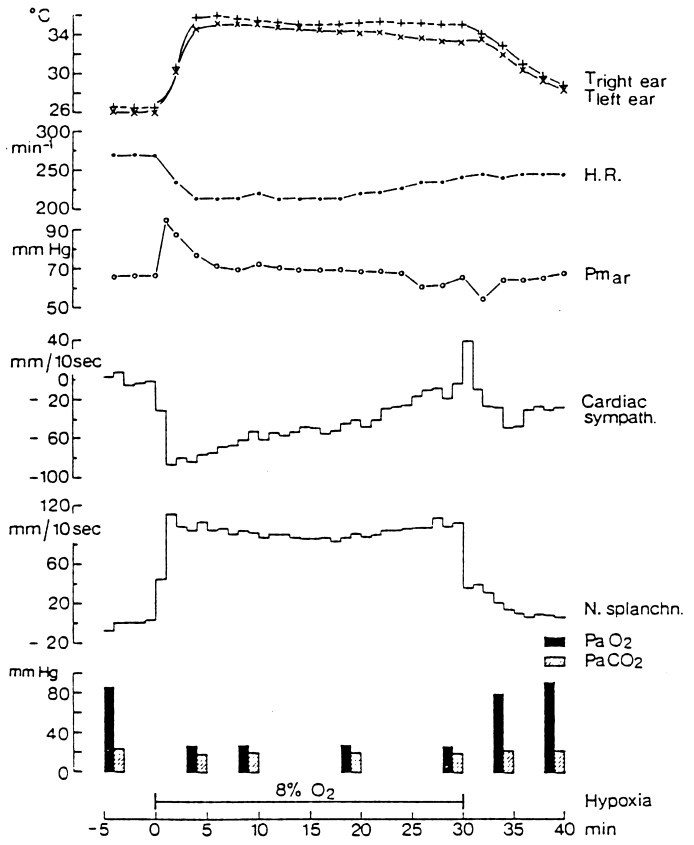

Fig. 4. Courses of ear skin temperatures (T left ear, T right ear), heart rate (H.R.), mean arterial pressure (P_{mar}), cardiac SNA, intestinal SNA, blood gas values during prolonged hypoxia (ventilation with 8% O_2 in nitrogen for 30 min) in a vagotomized, anesthetized rabbit [after Iriki *et al.* (1972)]. The increase in ear temperature indicates a decrease in SNA to the ear, a finding confirmed in their subsequent study (Iriki *et al.*, 1979).

6. DIFFERENCES IN SYMPATHETIC NERVE ACTIVITY BETWEEN ORGANS

Although it is not the focal point of this review it is important to reflect on the differential nature of the control of SNA. Indeed, while this is one of the hallmarks of circulatory control it is generally overlooked by many researchers who measure SNA to a single organ and then describe an effect of a treatment as increasing or decreasing SNA in general (Morgan *et al.*, 1995; Somers *et al.*, 1995). The SNA response to almost all stimuli is adjusted in a differential manner to different end organs, with quite clear differences between types of stimuli. In this way a tailored response to each stimuli can be produced. An excellent example of this characteristic is the response to moderate hypoxia in the rabbit where the overall cardiovascular response is one of little change in blood pressure. However, this belies a tremendous redistribution of blood flow. SNA is profoundly increased to the kidney and gut, but decreased to the heart and skin (Fig. 4). It should be stressed that this differential control system is likely to underlie a primary means of circulatory control in daily life. The origin of this control extends from distinct afferent pathways, to distinct central nervous system cells groups (McAllen and Dampney, 1990; McAllen and May, 1994; Polson *et al.*, 1995) and distinct spinal cord pathways (Edwards *et al.*, 1996; Pyner and Coote, 1994). This differential control is likely to work in concert with frequency patterning to different organs, thus the range of responses that can be configured to different stimuli is extremely complex. Some researchers have simultaneously measured SNA to several end organs such as the heart, kidney and muscle (Gebber *et al.*, 1994a; Kocsis *et al.*, 1990, 1993), and by measuring the similarities and differences between different pairs of nerves formulated a hypothesis of multiple coupled brain stem generators to explain the origin of certain rhythms (Gebber *et al.*, 1995b) (principally cardiac related and 10 Hz, see later). A limitation of this hypothesis is that some SNA frequencies may have different patterns to different organs, not because they reflect separate SNA generating mechanisms but rather as a reflection of the differential control over those frequencies. Generation of the underlying sympathetic rhythm may be quite a separate affair.

7. METHODOLOGY

While neurophysiologists have always considered the properties of individual sympathetic discharges to be important, researchers measuring SNA as an index of circulatory control have tended to ignore the rhythmical properties of discharges, preferring to average the signal over a time period between 1 and 10 sec (Dorward *et al.*, 1985; Iriki and Kozawa, 1983; Morgan *et al.*, 1995). Thus a single measurement of nerve activity which increases or decreases with various stimuli is obtained; this is termed overall SNA in this review. While it is true that this does give an index of the overall level of SNA it obviously ignores SNA's most striking feature, its rhythmicity. Indeed it seems incongruous given Adrian and colleagues (Adrian *et al.*, 1932) description of SNA as rhythmical in the 1930s and its easily identified characteristics from an auditory signal, that many researchers in the 1980s took the line that such information was not relevant.

Irrespective of the analysis approach, with regard to measuring SNA a range of different filtering settings for the original signal have been used. These include a band-pass of 1–1000 Hz in studies by Gebber and colleagues (Barman *et al.*, 1992; Gebber, 1976; Gebber and Barman, 1980).

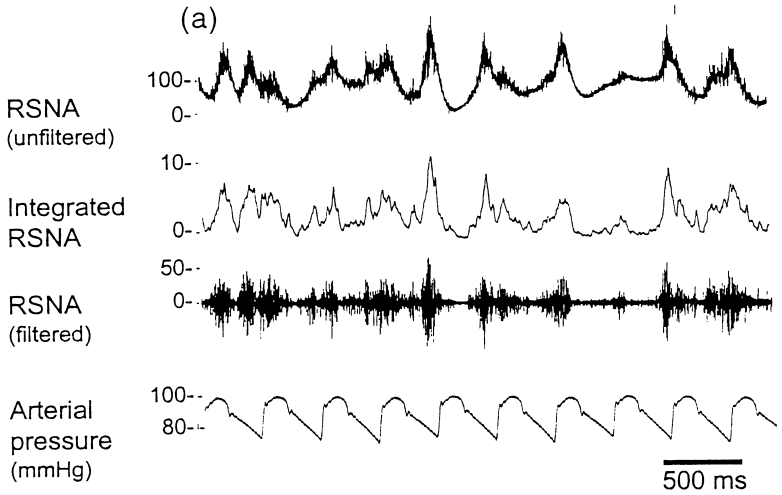

Fig. 5. Sympathetic discharge characteristics recorded from the renal sympathetic nerve of a chloralose-anesthetized cat. Two recording strategies are shown. The upper trace shows the signal recorded using a wide bandpass (1–3000 Hz) (RSNA unfiltered), where negative (upwards) waves represent envelopes of burst activity as used by Gebber and colleagues (Gebber, 1976). The next trace show the same neurogram recorded with a band pass of 100–1000 Hz and its integration (20 msec time constant, integrated RSNA) (Malpas and Ninomiya, 1992d). The next trace shows the neurogram filtered between 100–1000 Hz but no integration (RSNA filtered). After (McAllen and Malpas, 1997).

However, cardiac- and respiratory-related movement artifacts can adversely affect the signal when this wide bandpass is used. The neurogram tends to appear with a floating baseline with the cardiac and respiratory related discharges lain on top (Fig. 5). Others have excluded these DC like fluctuations by incorporating a greater highpass filter setting between 30 and 100 Hz (Kubota *et al*., 1995; Malpas *et al*., 1996; Malpas and Ninomiya, 1992a; Ootsuka *et al*., 1995). This removes the DC like nature of changes in the baseline and produces a baseline at 0 V with each discharge then showing a simultaneous deflection in a positive and negative direction. While it is possible to analyse this signal directly, in practice, since the action potentials from individual nerves cannot be extracted from this signal reliably (Andresen and Yang, 1989), the most common method is to apply a full wave rectification and short term integration to the signal (Fig. 5) (Kenney, 1994; Kenney and Fedde, 1994; Malpas and Ninomiya, 1992b). Using a time constant of 20 msec for the integrator produces a signal composed of a series of peaks or waves. This signal then is amenable to computer based acquisition and analysis of its rhythmical properties. Kenney (Kenney, 1994; Kenney and Fedde, 1994) has compared the SNA rhythmicity obtained using either the wide band pass cutoff frequencies (1–1000) or the narrower filter of 30–3000 Hz and found the spectra of SND are similar but with the advantage that the narrow-band amplification (30–1000 Hz) eliminates movement-related artifacts, which may adversely influence the sympathetic nerve signal.

Two main methodologies have been used to identify the various rhythmical components of SNA. The simplest is time domain analysis where the discharges are detected and the time interval between them measured (Kubota *et al*., 1995; Malpas and Ninomiya, 1992d). This approach is really only suitable for identifying rhythms above the respiratory frequency and where the discharges occur as easily identifiable peaks in the signal. The other approach is spectral analysis. This is performed by fast Fourier transform of the data to separate the frequency components in a signal and quantifies the proportion of the total activity contained with designated frequency bands. The relative strength of each rhythm is plotted against the frequency. While this technique involves some rather complex mathematics that can seem daunting to the uninitiated, its advantage lies in its ability to identify rhythms at multiple frequencies, in particular frequencies below the respiratory cycle and to determine the effect of one rhythm on another. Gebber and colleagues have primarily used spectral analysis for analysis of the faster rhythms associated with the cardiac cycle and above (see later) (Barman *et al*., 1992; Gebber and Barman, 1980). By conducting spectral analysis on activity recorded from pairs of nerves and performing cross spectral calculations to determine the interactions between pairs of nerves or the activity of cell groups and SNA they have made a range of proposals on the generation of SNA (see Section 8). It should be noted that the use of spectral analysis to analyse such frequencies does have some pitfalls. Firstly it does require a signal that is quite regular and stable (termed stationary). While SNA has some rhythmical properties, it is certainly not completely regular. Furthermore, while it may seem a simplistic argument, it does seem excessive to subject a signal to a complex analysis to determine its rhythmicity when this can easily be measured from interburst time intervals and plotting these intervals as a probability histogram (Kubota *et al*., 1995). A rather esoteric argument has occurred in the literature where Zhong *et al*. (1996) have argued that the construction of probability distributions for intervals between discharges may distort the rhythms present and that spectral analysis is the optimum method of analysis. They suggest that the observation of a mode of 100 msec in a probability distribution does not necessarily indicate a rhythm of 10 Hz. Overall, spectral analysis has much to offer the field, in particular for determining low frequency rhythms in SNA and interactions between rhythms to identify the sources of those rhythms, however, its usefulness may be overstated when some rhythms can easily be seen through simple time domain methods.

8. WHAT RHYTHMS ARE PRESENT IN SYMPATHETIC NERVE ACTIVITY?

In this review the term rhythmicity can imply that SNA discharges display a fixed cycle length in their discharge properties. SNA while displaying rhythmical aspects that can be measured using tools such as spectral analysis and simple time domain analysis, is also inherently irregular in a number of aspects. Predominantly these are in the amplitude of discharges and in the cardiac related nature of the discharges and these are discussed in the appropriate sections. Furthermore, although sympathetic tone is a common feature to all animals serving to maintain arterial pressure, it must be noted at the outset that the rhythmicity is state dependent. That is, that certain rhythms may appear, disappear or increase in their strength during certain stimuli (DiBona and Jones, 1995; Häbler *et al*., 1993; Kocsis and Lenkei, 1992). This makes it difficult to compare results obtained from different experimental conditions, including conscious, decerebrate and anesthetized. Notwithstanding this limitation several frequencies have been identified.

9. CARDIAC (2–6 HZ)

When all the various rhythmicities in SNA are identified and their relative strengths compared using power spectral analysis, the majority of the spectral power is located at frequencies >3.5 Hz, and in particular between 3.5 and 5 Hz [53% on average for renal SNA in the conscious rabbit (Janssen *et al*., 1997) (Fig. 6)]. Ever since it was observed in the first recording by Adrian *et al*. (1932) it has been the subject of considerable research as to its origin. Initially it was thought that the 2–6 Hz rhythm simply reflected inputs from baroreceptors to vasomotor centres, as at about the same time it had been shown that baroreceptor

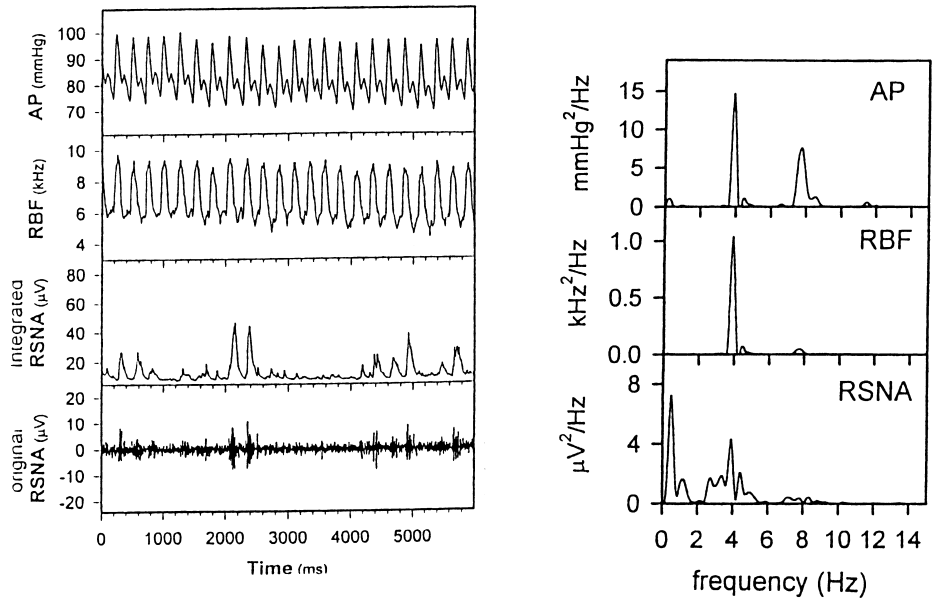

Fig. 6. Left panel, trace of arterial pressure (AP), renal blood flow (RBF) and renal sympathetic nerve activity (RSNA) in a conscious rabbit. Right panel, corresponding spectral density power calculated. In the plot of AP, a peak at twice the cardiac frequency was found because of pulse wave reflection. Adapted from (Janssen *et al.*, 1997).

nerves also displayed a cardiac related signal (Bronk and Stella, 1932). It had already been established that sympathetic outflow depended on connections from the brainstem (Dittmar, 1873). Increased baroreceptor nerve discharge during systole was thought to travel to the brainstem and cause direct inhibition of sympathetic preganglionic nerves, whereas reduced baroreceptor nerve discharge during diastole was considered to lead to increased SNA via disinhibition. In this way it is possible to explain how the timing of discharges were related to the heart rate. This simple hypothesis, however, could not account for discharges at greater than the heart rate, indeed even in early work when blood pressure was lowered one can see signs that reducing baroreceptor input gradually changed sympathetic activity from regular cardiac related discharges to a more continuous pattern (Bronk *et al.*, 1936) (Fig. 7). This observation is pertinent when discussing the changes in this rhythm under baroreceptor denervation later in this section. By the 1960s it was realized that baroreceptors could not provide the sole explanation for the rhythmicity of SNA. Green and Heffron (1967) observed a rhythm faster than the heart rate (up to 10 Hz) during asphyxia or reductions in blood pressure. They did not go as far as to suggest that the cardiac rhythmicity was of central origin, but rather suggested that there was a fast rhythm, produced by a medullary generator, which 'pushed' through to overlie the cardiac rhythm of baroreceptor origin when central drive was enhanced. The baroreceptor origin hypothesis was supported by their later observations of cardiac SNA in cats, in which the baroreceptors had been isolated from the systemic circulation (Green and Heffron, 1968). When an artificial pulsatile input was applied to the baroreceptors, SNA also assumed the rhythm of the pump. However, it should be noted that the results of these studies were based solely on observations of the neurograms rather than on more detailed analysis as subsequent studies have been.

The above observations would predict that baroreceptor denervation would reduce the occurrence of cardiac related sympathetic discharges while other rhythms might remain, such as at 10 Hz. It was therefore a surprising observation that after baroreceptor denervation the rhythmicity was to some extent maintained, although no longer in phase with the cardiac cycle (Barman and Gebber, 1980; Taylor and Gebber, 1975) (Fig. 8). They suggested that a 2–6 Hz rhythm in SNA was representative of a sym-

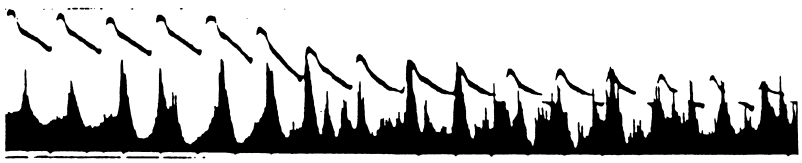

Fig. 7. Original neurogram from a multifibre nerve recording of cardiac SNA (bottom panel) in the anesthetized cat (top panel is arterial pressure, both signals were uncalibrated). Note the pulse-grouping of discharges at the start of the trace, and the change to a more continuous discharge pattern as pressure falls. After (Bronk *et al.*, 1936).

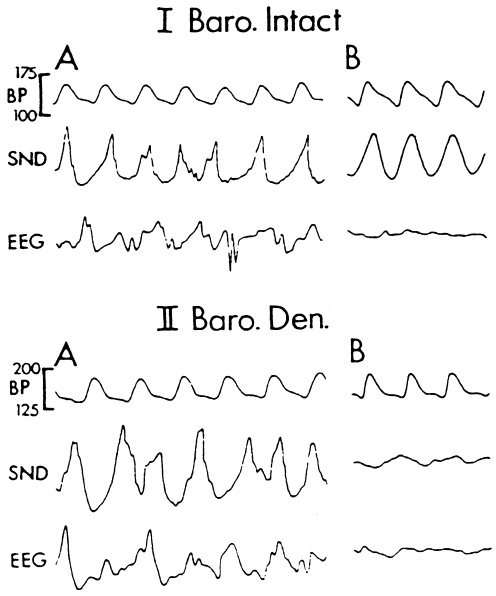

Fig. 8. (A) Original neurograms of splanchnic SNA and EEG before and after baroreceptor denervation. BP is measured in mmHg (top), splanchnic SNA (middle) and parietal-frontal EEG (bottom). Negativity is recorded as an upward deflection. (B) R wave-triggered computer averaged records of the traces from (A). Note that after baroreceptor denervation although SNA remained rhythmical it no longer discharged in phase with the cardiac cycle. After Barman and Gebber (1980).

pathetic rhythm of central origin that normally is entrained to the cardiac cycle by phasic baroreceptor input (McCall and Gebber, 1975). Several lines of convincing evidence were proposed to support this hypothesis besides the effect of baroreceptor denervation; firstly, a sympathetic discharge could be aborted by a single shock delivered to baroreceptors early in the cardiac cycle, even though that shock comprised no greater than 1% of the duration of the cardiac cycle. Secondly, the phase relationship between the arterial pulse and the sympathetic discharge could be reset by slowing the heart rate. These observations had the effect of transforming the overall area from one of somewhat esoteric interest into an area of profound neurophysiological interest, with a search for cell groups and pathways involved in producing and controlling these rhythms.

9.1. Central Mechanisms Generating Sympathetic Discharges

Two main proposals have been advanced to account for the inherent rhythmicity of SNA; the first is that there is a single solitary brain stem oscillator or pacemaker, the second is that there is a network of cell groups whose combined action provides the inherently rhythmical activity. In any case the neural structures responsible for generating sympathetic tone are believed to be located in the medulla, since midcollicular or midbrain transection generally resulted in little reduction of arterial pressure (Dittmar, 1873). With regard to the pacemaker theory it has been proposed that the activity of rostral ventral lateral medullary cells (RVLM) forms the basis of this phenomenon (Sun *et al.*, 1988a,b). These neurons project to the spinal cord and are inhibited by baroreceptor inputs, indicating that they are sympathoexcitatory. Some of these neurons seem to generate action potentials by autodepolarizations (gradual depolarizations), thus showing spontaneous activity (Li and Guyenet, 1996, 1997; Sun, 1995). Recordings of intracellular activity from medullary slices have shown the presence of ramp-like depolarizations following each action potential leading to subsequent action potentials with no evidence of excitatory postsynaptic potentials (EPSPs) (Li *et al.*, 1995; Li and Guyenet, 1996). Furthermore, the regular firing pattern of these cells (between 5 and 30 Hz in the slice preparation) could be reset after short depolarizing current pulses (Sun *et al.*, 1988a,b) and injection of the GABA agonist, muscimol into the RVLM while reducing the overall level of SNA, did not alter the SNA rhythmicity. Indeed a combination of excitatory amino acid and GABA receptor blockade into the RVLM blocked a large number of reflexes (e.g. baroreceptor) but did not alter the rhythmicity of SNA (Guyenet *et al.*, 1987; Sun and Guyenet, 1986a). This suggests that some RVLM neurons have an intrinsic activity. It was proposed that synaptic and other extrinsic factors only modulate the basic rhythm generated by these neurons. One of the main objections to this theory is that the average discharge rate of these pacemaker cells is much higher [averaging 8.6 spikes sec^{-1}, Li and Guyenet (1995)] than that seen in SNA [normally between 1 and 6 Hz, Malpas *et al.* (1996)], and no mechanism has been advanced to explain how such frequencies could be converted to a slower frequency rhythm by periodic baroreceptor input. Furthermore, intracellular recordings of RVLM activity *in vivo* by Lipski *et al.* (1996) have shown that RVLM neurons, while sometimes spontaneously active, display largely an irregular pattern of firing. They also showed that the ongoing activity (both EPSPs and IPSPs) resulted from synaptic inputs and did not display typical activity that might be expected from an intrinsic pacemaker system, that is, there was no evidence for gradual depolarizations between individual action potentials. They have suggested that the regular pattern of activity observed in tissue slices 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 presympathetic neurons under standard conditions in vivo. The work of Lipski and colleagues is consistent with the 'network' hypothesis for the generation of SNA (Gebber, 1990). This implies that the activity of presympathetic neurons depends on their antecedent excitatory inputs. They suggest that the on-going activity of presympathetic neurons is controlled by tonic inhibitory input which is responsible for setting the level of excitability of these neurons.

With regard to the network hypothesis there are several questions; what is the role of baroreceptor input in the generation of SNA? Is a single system responsible for the range of frequencies present in SNA? How can the differences in SNA to different target organs be explained? What cell groups are

involved in the generation of the various rhythmicities? The model proposed by Gebber, Barman and colleagues suggests that brain stem cell groups are inherently capable of generating a rhythm that closely matches the heart rate but that without pulsatile input from baroreceptors this is free running (Gebber, 1990). This concept is based mainly on two observations; the timing between baroreceptor activity and sympathetic nerve discharges can be shifted by changing the heart rate, and the persistence of a rhythm after baroreceptor denervation (Barman and Gebber, 1980; Gebber, 1976; Gebber and Barman, 1980).

9.2. Role of Baroreceptors

Research in the 1970s and 1980s firmly established that arterial baroreceptors could not be the source of the 2–6 Hz rhythmicity in SNA, although they clearly provided an input to central generating mechanisms to induce discharges to occur at a certain phase of the cardiac cycle (Barman and Gebber, 1980; Gebber, 1976). It is important to note that the term cardiac related rhythm does not simply refer to the occurrence of discharges some time in each cardiac cycle but refers to the observation that the discharges occur at certain times in the cycle. Thus, while the number of discharges per minute may increase or decrease, the discharges that do occur happen with high probability at certain times of the cardiac cycle. In the rabbit the average time between systolic pressure and the maximal renal SNA voltage from an integrated burst is 76 msec (unpublished observations).

Gebber, Barman and colleagues central hypothesis is that a central oscillatory network is entrained by the pulse synchronous baroreceptor afferent nerve traffic (Gebber, 1990). That is, that rhythmical input from baroreceptors provides a positive and entraining effect on the centrally generated rhythm, that is, being 'kicked' along by input from baroreceptors (Gebber, 1976). One of the foundations of their hypothesis is that the temporal relationship between baroreceptor nerve activity and sympathetic nerve discharge can be dramatically shifted by changing heart rate. Slowing of the heart changes the interval between peak activity in carotid sinus afferents and renal SNA, but this interval should not have changed if the cardiac related rhythm in SNA was a simple consequence of the waxing and waning of central inhibition by baroreceptor inputs. However, the problem with this form of analysis is that the timing was conducted from the carotid sinus activity to the maximum SNA level. Baroreceptors do not provide phasic excitatory input but rather a phasic inhibitory input (Kumada *et al.*, 1990). Thus it is possible to argue the appropriate measure is that between the heart beat and the time of inhibition of a SNA discharge.

Hedman *et al.* (1994) have recently examined the relationship between the cardiac cycle and the timing of sympathetic discharges when the cardiac interval was changed by artificial pacing of the heart. When the delay time from the peak systolic pressure to the minimum cardiac SNA voltage was measured, they found that irrespective of cardiac intervals between 250 and 600 msec, the delay time was remarkably constant. Furthermore, they also found that after baroreceptor denervation the cardiac related rhythmicity was completely lost and faster rhythmicities prevailed. Recent observations using electrical stimulation of the aortic depressor nerve in rabbits with pseudorandom sequences while recording the SNA responses, have identified a fixed time delay of *ca* 400 msec irrespective of the input frequency for the central arc (Kubo *et al.*, 1996). In order to reconcile these observations with the earlier findings showing variable latencies as the heart rate changed (Gebber, 1976), it is necessary to propose that while the time until maximum inhibition of SNA by baroreceptors is of constant interval (Hedman *et al.*, 1994), the recovery process until the maximum activation of SNA is not constant. This suggests that the recovery from inhibition is a different process, governed by a different set of as yet undefined rules. These observations are different from Gebber and colleagues original premise and suggest the periodic input from the baroreceptors must provide *not* an entraining signal to the brain stem circuits but rather a phasic inhibitory signal, and that this is of a constant length, although the recovery may vary according to heart rate. Thus as the cardiac interval lengthens, the time until the onset of the next sympathetic discharge remains constant but the time until the peak activity may vary. One model proposed is that baroreceptors directly affect the sympathetic discharge generator, rather than simply modifying the transmission of its output (McAllen and Malpas, 1997). This would be able to explain the full extent of baroreceptor activity on the timing of discharges which extends from several discharges per second down to one burst every 10 or 20 heartbeats (Malpas *et al.*, 1996; Sundlöf and Wallin, 1978). If baroreceptors affect discharge generation, the generator must be 'downstream' of where baroreceptor signals meet neural pathways that determine sympathetic drive.

There have been several observations that baroreceptor denervation alters the mean frequency of discharges with the cardiac related frequency being replaced by higher frequency discharges (Hedman *et al.*, 1994; Malpas and Ninomiya, 1992c; Ninomiya *et al.*, 1990). Such changes tend to exclude a free running oscillatory system after baroreceptor denervation as proposed by Gebber and Barman (Barman and Gebber, 1980; Gebber, 1990; Gebber and Barman, 1980). It could be argued that baroreceptor denervation is an artificial arrangement which may under different anesthesia and experimental conditions produce different effects on SNA. However, Toda and colleagues used a system of non-pulsatile systemic circulation in goats to remove the periodic input from baroreceptors and also found an increase in the frequency of discharges to 8–12 Hz (Toda *et al.*, 1996). Re-examination of the data presented by Gebber and Barman suggest that even within their recordings the effect of baroreceptor denervation on sympathetic rhythms is by no means as clear as they had initially suggested. Firstly, the frequency of the rhythm that they indicate persists after denervation, is between 2 and 6 Hz (Barman and Gebber, 1980). This is in fact a

threefold range of frequencies and cannot really be defined as a distinct rhythm. Power spectral analysis of SNA before baroreceptor denervation shows a reasonably sharp peak at the cardiac cycle, however, after denervation the peak is broad and without preferred frequencies (Fig. 9). It has been proposed that such aperiodic behaviour is very unlike any known biological oscillator (Bachoo and Polosa, 1987a). Secondly, the initial studies of Gebber and colleagues used urethane or chloralose anesthesia (Gebber, 1976; Gebber and Barman, 1980), which they subsequently identified as depressive for the higher frequency components of SNA (Barman *et al.*, 1992, 1995a). Thus it may not have been possible to observe a shift to higher frequencies after baroreceptor denervation in their early studies.

It should be noted that the synchronization of nerve action potentials does not disappear during baroreceptor denervation or other physiological stimuli. The exception to this is cerebral ischemia (Kocsis *et al.*, 1993). Thus, while the rhythmicity of the discharges may be altered, their propensity to synchronize and fire together is intrinsic to the system. This raises the possibility that the control over the synchronicity of bursts is separate from that mechanism which regulates the timing of the discharges. In support of this, while intracisternal injection of the glutamate antagonist, kynurenate, reduced overall SNA and blocked baroreceptor reflexes, it did not reduce the synchronicity of discharges (Gebber *et al.*, 1989).

9.3. Multiple or Single Oscillators?

Rhythmic modulation of sympathetic discharges between 2 and 6 Hz (cardiac related) is a common feature of SNA observed in many different sympathetic efferents and cross spectral analysis reveals a high degree of coherence between nerve activities at this frequency (up to 80% with regard to cardiac and renal nerve activities (Kocsis, 1994, 1995)). This has been taken by some groups to indicate that this rhythm is originating from common or related structures in the central nervous system which are coupled together (Gebber and Barman, 1980; Kocsis *et al.*, 1990). One may take a simplistic approach to argue against this that since overall SNA can be differentially controlled to separate organs then it must be a product of selective central pathways. For example, the neuroanatomical marker of immediate–early gene expression c-*fos*, under different stimuli shows quite distinctive patterns with activation to different stimuli (Hirooka *et al.*, 1997; Polson *et al.*, 1995). Or one might suggest that it is possible to have completely separate pathways each receiving some common afferent inputs and that various stimuli increase or decrease the strength of this input similarly to second order neurons. However, a system of coupled brain stem oscillators which drive separate groups of preganglionic neurons has been proposed (Gebber and Barman, 1980). Under this arrangement the multiple oscillatory systems receive some common inputs, such as from baroreceptors, and also some distinctive inputs. The function of this coupling may act as a stabilizing factor preventing disproportionate regional sympathetic reactions (Kocsis, 1994). Two models have been proposed to explain the nature of coupled oscillators; in the first model the multiple circuits responsible for SNA to different organs interact with each other to a variable degree. In the second model there are multiple circuits which receive identical inputs but which filter the inputs to a variable extent. In support of the first model, three relationship patterns between the discharges of sympathetic nerve pairs in baroreceptor denervated cats have been identified (Kocsis *et al.*, 1990). The interval can be constant, frequency dependent or uncoupled. They propose that this represents different states of the circuits involved in the control of the 2–6 Hz rhythm in SNA and that this

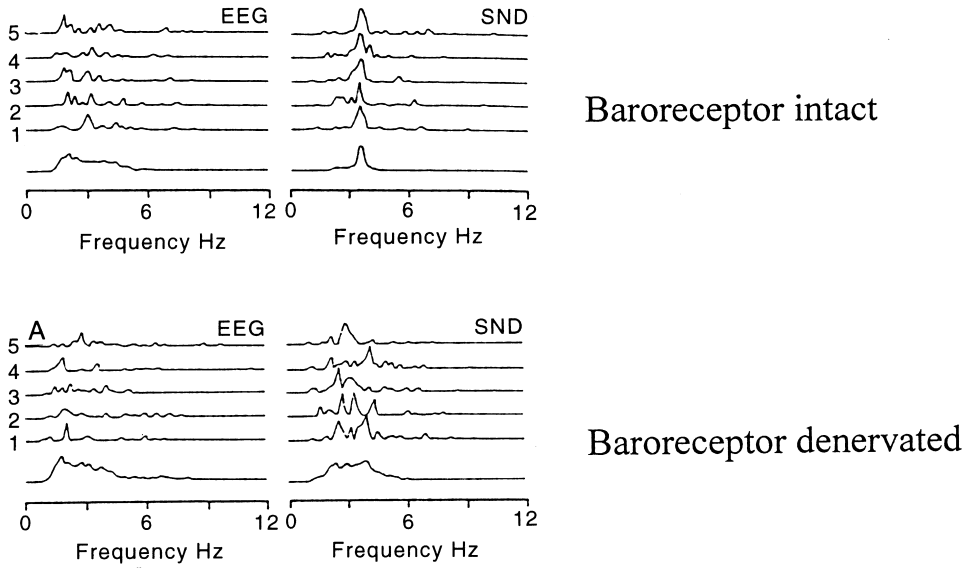

Fig. 9. Power spectra of splanchnic SNA before and after baroreceptor denervation in a chloralose anesthetized cat. After Barman and Gebber (1980).

relationship is affected by different stimuli such as asphyxia and haemorrhage (Kenney, 1994). At present there is little experimental information regarding the second model.

9.4. Central Pathways Involved in Generating Sympathetic Rhythms

It is agreed that neurons in the RVLM play a critical role in the maintenance of the basal level of sympathetic discharges and thus arterial pressure (Dampney, 1994b; Dampney *et al*., 1982). Indeed their destruction reduces arterial pressure to a similar level to that seen after spinal cord transection (Dampney and Moon, 1980). It is therefore natural that much of the research in this area has focused on the role of this group of neurons in regulating the rhythmicity of SNA. It is unanimously agreed that the RVLM participates in the arterial baroreflex and barosensitive neurons have been found in the RVLM of cats (Caverson and Ciriello, 1984), rats (Brown and Guyenet, 1985) and rabbits (Terui *et al*., 1987). Baroreceptor stimulation results in an inhibition of spontaneous discharges of RVLM neurons (Barman and Gebber, 1983; McAllen, 1986; Terui *et al*., 1987). This effect is transmitted via the nucleus Tractus Solitarius and the caudal ventrolateral medulla (Dampney, 1994a). The majority of studies indicate that there is disfacilitation of neurons in the RVLM by baroreceptors. However, while the RVLM is an important source of tonic sympathetic activity it is unlikely to be the sole source of the rhythmicity of sympathetic discharges as blockade of all synaptic transmission in the RVLM leads to a reduction in the power of the 2–6 Hz rhythm of SNA without reducing overall SNA levels (Trzebski and Baradziej, 1992). In order to produce such a differential response it is necessary to assume that SNA becomes desynchronized. Therefore while the RVLM may not generate bursts it may regulate the synchronization of the resulting activity.

Studies of Gebber, Barman and colleagues have measured unit activities and population field potentials of neurons correlated to the 2–6 Hz rhythm in SNA using coherence analysis (Barman and Gebber, 1997; Barman *et al*., 1994b, 1995b; Huang *et al*., 1992; Montano *et al*., 1996). These include the RVLM, the caudal ventrolateral medulla (CVLM), the caudal raphe nuclei, the lateral tegmental (LTF), the pontine parabrachial and the Kölliker–Fuse complex. All of these structures have been proposed to contribute to the network generation of SNA discharges. Several lines of evidence indicate the LTF of the dorsal reticular formation as a contender for producing rhythmical input to the RVLM. The naturally occurring discharges of the LTF neurons are synchronized to the cardiac-related rhythm in postganglionic SNA of both baroreceptor innervated and denervated cats (Montano *et al*., 1996). LTF neurons discharge earlier in the 2–6 Hz oscillation in SNA than the RVLM neurons (Barman and Gebber, 1993; Gebber and Barman, 1985). The axons of the LTF sympathoexcitatory neurons (those inhibited by baroreceptor reflex activation) project to the region of the RVLM that contains sympathoexcitatory neurons innervating the thoracic intermediolateral nucleus (Barman *et al*., 1995b). Finally they can be antidromically activated by stimulation of the RVLM and RVLM neurons can be activated by stimulation of the LTF (Barman and Gebber, 1987). Thus there is likely to be a reciprocal input back to the LTF from the RVLM, introducing the possibility of a feedback mechanism controlling the 2–6 Hz rhythm. However, the evidence is not all in favour of the LTF regulating rhythmicity in SNA as bilateral muscimol injections into the LTF does not change cardiac related SNA (Barman and Gebber, 1993) and retrograde tracer studies show the LTF has only a few neurons that project to the RVLM (Dampney *et al*., 1987). There is also evidence that cells in the pontine reticular formation (PRF) provide tonic excitatory drive to some cardiovascular neurons located in the RVLM as glycine injection into the PRF eliminated or reduced the ongoing activity of cardiovascular units in the RVLM (Hayes *et al*., 1994).

9.5. What is the Role of Direct Spinally Projecting Neurons?

While the importance of the RVLM in maintaining sympathetic tone is established, the role of other cell groups which have direct projections to the spinal cord, in regulating the rhythmicity of SNA has not been explored. Examples include the paraventricular nucleus (PVN) of the hypothalamus and the A5 cell group of the pons, both of which provide a major component of the inputs to preganglionic neurons in the spinal cord (Strack *et al*., 1989b). These cell groups play a role in the regulation of blood volume (Lovick *et al*., 1993; Martin *et al*., 1997) and the sympathetic response to chemoreceptor stimulation, respectively (Koshiya and Guyenet, 1994). Changes in the rhythmicity of SNA during activation of each of these sites would have great bearing on our understanding of the mechanism's generating sympathetic discharges. If their activation altered the frequency of discharges, this would suggest modulation of discharge generation at a spinal site (not withstanding non-direct connections). This action may be via a simple filter to already formulated discharges or may actually produce new discharges. Alternatively, activation may regulate the number of nerves recruited by acting as a gain control, increasing or decreasing the number of recruited preganglionic neurons without changing their frequency of firing (see later section on the amplitude of sympathetic discharges). In support of this hypothesis stimulation of the PVN leads to increases in the amplitude of sympathetic discharges without change in their frequency of firing (Malpas and Coote, 1994). Observations in anesthetized animals that extracellular activity at both these sites is normally silent (Byrum *et al*., 1984; Lovick and Coote, 1988), suggest they do not influence the resting generation of bursts, although the situation in the conscious animal may be different.

10. RESPIRATORY

The presence of a respiratory rhythm in SNA was first shown in 1932 by Adrian *et al.* (1932). The particular type of rhythmicity seen can differ between species (Häbler *et al.*, 1994). In general sympathetic neurons are activated during inspiration although the precise timing of this activation differs to different organs. SNA to the heart, kidney and adrenal has an early inspiratory activation with a postinspiratory or early expiratory dip, while the cervical and lumbar nerve activities have a postinspiratory activation (Boczek-Funcke *et al.*, 1992b; Numao *et al.*, 1987). Muscle vasoconstrictor nerves appear to have a strong inspiratory activation while sudomotor neurons have an expiratory peak. Furthermore sympathetic preganglionic neurons to the gut have no respiratory modulation at all (Häbler *et al.*, 1994).

Because blood pressure oscillates with a respiratory cycle, to some extent the strength and phase of the respiratory rhythm is a product of reflex modulation (Boczek-Funcke *et al.*, 1992a). In other words, ventilation itself causes changes in intrathoracic pressure which in turn affects venous return, cardiac output and arterial pressure, which activates both arterial and cardiopulmonary baroreceptors. Certainly in the case of an artificially ventilated animal the changes in arterial pressure with respiration can be quite large. However, it is also clear that such mechanically induced changes cannot account for differences in the rhythm between SNA to different end organs some of which, for example, sudomotor, show little baroreceptor modulation (Jänig, 1988). Furthermore, artificial ventilation combined with a pneumothorax and vagotomy removes the afferent lung inflation signals and baroreceptor mediated oscillation in arterial pressure, yet SNA still contains a respiratory signal that occurs out of phase with the ventilator (Miyawaki *et al.*, 1995)..

The origin of inherent respiratory rhythm in SNA is thought to be due to a central coupling between respiratory neurons and neurons of autonomic pathways (Häbler and Jänig, 1995; Pilowsky, 1995). The respiratory rhythmicity in SNA persists in immobilized animals during asphyxia, induced by temporarily stopping the artificial ventilation (Tang *et al.*, 1957). The magnitude of the respiratory modulation is proportional to respiratory drive (Fig. 10). Hypercapnia enhances it, while hyperventilation decreases the sympathetic activation during inspiration (Barman and Gebber, 1976; Boczek-Funcke *et al.*, 1992b; Cohen and Gootman, 1970; Millhorn, 1986). By studying the respiratory modulation of lumbar sympathetic nerve discharge in anesthetized, paralysed and vagotomized rats during microinjection of GABA antagonists into the RVLM, Guyenet and colleagues have suggested that the critical link between the central respiratory rhythm generator and sympathetic outflow lies at the rostral tip of the ventrolateral medulla (Guyenet *et al.*, 1990; Koshiya and Guyenet, 1996). Neurons of the ventral respiratory group, including the Bötzinger and pre-Bötzinger cell groups, have a very prominent respiratory modulation of their ongoing activity (Smith *et al.*, 1991b), but while modulation of barosensitive bulbospinal neurons in the RVLM is less obvious, a postinspiratory activation has been identified (Haselton and Guyenet, 1989). Triggering by phrenic nerve activity to produce histograms shows at least five different types of respiratory modulation in RVLM neurons (Miyawaki *et al.*, 1995; Pilowsky, 1995). It is not clear what purpose this range of patterns has but it may be responsible for the different respiratory rhythms seen in SNA to different end organs. It has been proposed that the inspiratory neurons of the ventral respiratory group provide the respiratory modulation to bulbospinal neurons and thus SNA (Pilowsky, 1995). Tracers injected into respiratory neurons have been transported to axon terminations in the area of the bulbospinal neurons and in particular the subretrofacial nucleus of the RVLM (McAllen, 1986, 1987). Neurons generating central respiratory activity are located in the ventrolateral medulla just dorsal to and intermingled with the cardiovascular neurons (Lipski and Merrill, 1980; Pilowsky *et al.*, 1990). Anatomically therefore, there is a close association between centres involved in the control of

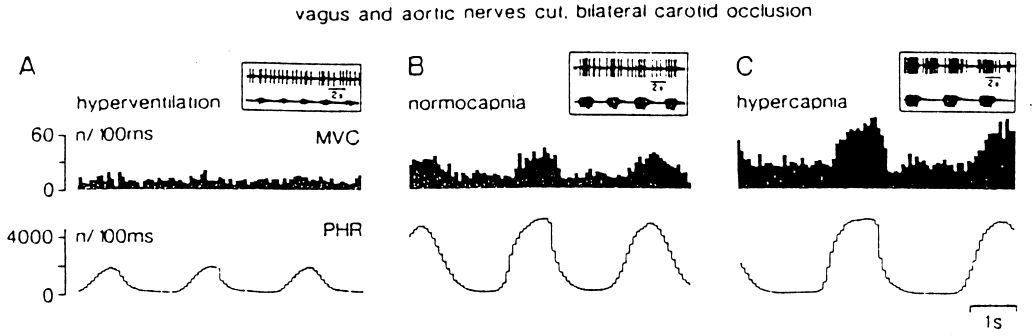

Fig. 10. Typical respiratory modulation (central component) of the activity in a single preganglionic neuron projecting to the cervical sympathetic trunk of an anaesthetized cat. The animal was bilaterally vagotomized and the arterial baroreceptors were functionally eliminated by bilateral occlusion of the carotid arteries. Artificial ventilation was with positive pressure. Histograms were triggered with the rapid decline of phrenic nerve activity, bin width 100 msec, and averaged over 100 respiratory cycles averaged. Insets show original signals (upper trace) and phrenic activity (PHR). After Häbler *et al.* (1994) and adapted from Boczek-Funcke *et al.* (1992b).

respiration and those involved in sympathetic activity.

At least three models have been proposed to explain the respiratory related rhythm in SNA that exists in vagotomized animals. The neurons of the respiratory oscillator may impose their rhythm on the circuits that control SNA (Bachoo and Polosa, 1986, 1987b). Alternatively there may be a common respiratory/sympathetic generator, which receives inputs from a common oscillator while containing some separate circuits (Richter and Spyer, 1990). In the third model, separate generation and control of respiratory and sympathetic rhythms exist but these are normally coupled (Barman and Gebber, 1976). There are several reasons why a common respiratory/sympathetic generator is unlikely. Firstly Barman and Gebber have observed that changes in respiratory rate were accompanied by dramatic shifts in the phase relation between sympathetic and phrenic nerve discharges (Barman and Gebber, 1976) (Fig. 11). They also observed the respiratory oscillations of sympathetic and phrenic nerve discharge were not always locked in a 1:1 relation. Furthermore, they showed hyperventilation causing quiescence of phrenic activity did not remove a respiratory rhythm in SNA. They suggested that the respiratory components of SNA and phrenic nerve activity are generated independently of each other but that they are normally entrained to each other. This degree of coupling must be quite plastic in order to account for the different levels of rhythmicities seen under different ventilatory states. Support for a coupled oscillator concept has recently come from measurement of the respiratory rhythm in pairs of sympathetic nerves after mathematical removal of the portion of the signal in phase with the phrenic nerve activity (Zhong *et al.*, 1997). SNA continued to show some residual respiratory rhythmicity after phrenic activity was eliminated during hypocapnia.

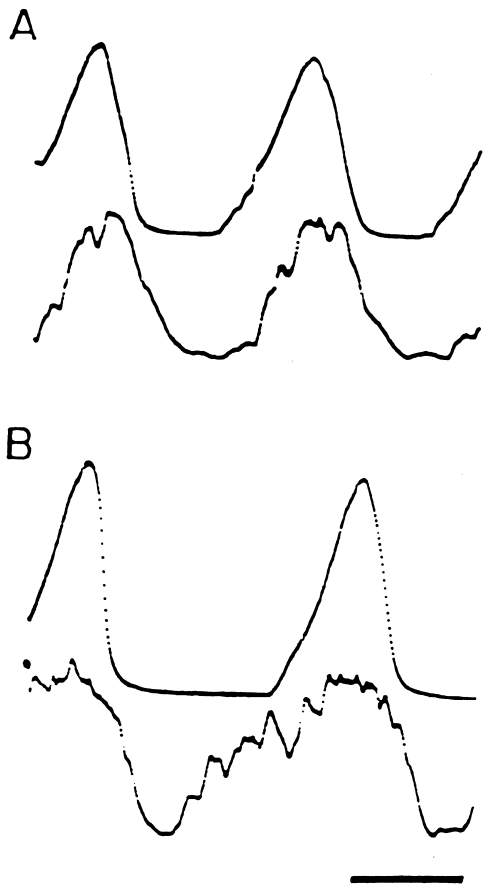

Fig. 11. Shift in phase relations between phrenic and sympathetic nerve discharge accompanying change in respiratory rate in vagotomized cat. Phrenic trace, top; cervical sympathetic activity trace bottom. Respiratory rate was: (A) 31 cycles min^{-1} and (B) 24 cycles min^{-1}. Horizontal calibration is 1 sec. After Barman and Gebber (1976).

11. THE 10 HZ RHYTHM

This is currently probably the most debated rhythm within SNA. It was first observed by Green and Heffron (1967) and identified as 10 Hz in splanchnic nerve activity from anesthetized cats in 1970 (Cohen and Gootman, 1970). Subsequently it was 'forgotten' until 'rediscovered' by Ninomiya *et al.* (1990). The terms forgotten and rediscovered are appropriate here as research by Gebber, Barman and colleagues in the 1980s made no mention of the rhythm in their many papers (Barman and Gebber, 1980, 1983; Gebber, 1990; Gebber and Barman, 1980). This oversight may in part be due to the use of diallylbarbiturate–urethane or chloralose anesthesia which seems to mask the 10 Hz rhythm. To some extent the description as a 10 Hz rhythm suggests a highly regular signal with a period of 100 msec. In fact the rhythm has a reasonably wide frequency band between 8 and 12 Hz (Ninomiya *et al.*, 1990). This rhythmic activity seems to have no correlation with other internal cycles (Barman *et al.*, 1995a) and its probability of occurrence is quite variable. It appears to be enhanced when the overall SNA level is increased, such as in the inspiratory phase (Cohen and Gootman, 1970) or after baroreceptor denervation (Malpas and Ninomiya, 1992c).

Ninomiya and colleagues proposed that the 10 Hz rhythm is related to the cardiac cycle and hypothesized it was a fundamental rhythm of an oscillator and that gates opened and closed to produce the cardiac related rhythm (Ninomiya *et al.*, 1990). The opening and closing of the gates was controlled by baroreceptor inputs. They supported this hypothesis with data showing the 10 Hz rhythm was a ratio of the cardiac related rhythm, that is, that the gates would remain closed for one, two or three cycles of the 10 Hz oscillator to produce a burst that was cardiac related. In support of this, baroreceptor denervation is generally associated with a shift to higher frequency discharges (Malpas and Ninomiya, 1992c). However, subsequent analysis of other data found the cardiac related discharges were a ratio with the 10 Hz rhythm no more often than would be expected by chance, rather it was suggested that the cardiac related rhythm and the 10 Hz rhythm are the product of different controlling and generating systems (Malpas, 1995).

Table 1.

Neurons with a relationship to the 10 Hz rhythm	Type of relationship
CVLM (Barman *et al*., 1995b)	Neuronal activity correlated to the 10 Hz rhythm (fires before raphe neuron)
Lateral tegmental field (Barman and Gebber, 1993)	Neuronal activity not correlated to the 10 Hz rhythm but affects the occurance to the 10 Hz rhythm in SNA
RVLM (Barman and Gebber, 1997)	Neuronal activity correlated with a 10 Hz rhythm in SNA
Raphe neurons (Barman and Gebber, 1992, 1997)	Coherence between neuronal activity and the 10 Hz rhythm in SNA
Pontine parabrachial and Kolliker–Fuse complex (Zhong *et al*., 1992)	Lesion reduces 10 Hz rhythm but not 2–6 Hz rhythm. Neuronal activity not correlated with a 10 Hz rhythm in SNA

Gebber, Barman and colleagues have undertaken a number of studies to try and pinpoint the source of the 10 Hz rhythm and its control (Table 1). They have observed a high degree of coherence in the 10 Hz frequency between pairs of nerves before and after baroreceptor denervation (Barman *et al*., 1992). This coherence was stronger than that seen in the 2–6 Hz band. Furthermore the observation that the 10 Hz rhythm was not a harmonic of the cardiac related 2–6 Hz rhythm and essentially unrelated (Barman *et al*., 1992), also supports the above hypothesis that the two rhythms are generated independently (Malpas, 1995). The 10 Hz rhythm appears to be eliminated by knife cut through the medullary raphe nuclei suggesting its origin is above this level (Zhong *et al*., 1992, 1993a). They also suggested the RVLM was involved in its control as these neurons showed activity that was correlated with a 10 Hz rhythm in SNA (Barman and Gebber, 1997). This would not have been identified by simple measurement of the mean firing rates of the neurons as previously conducted, which show activity between 3 and 4 Hz in the cat (McAllen, 1986). Coherence between neuronal activity and the 10 Hz rhythm in SNA was observed for the caudal medullary raphe neurons (Barman and Gebber, 1992, 1997). They also predicted that since the discharges of the medullary neurons were correlated to the sympathetic discharge after the unit spike occurrence, then the 10 Hz rhythm was unlikely to be generated in the spinal cord (in that case the medullary activity would have correlated to the sympathetic discharge preceding the unit spike). In their recent work they suggest that there is convergence of the 10 Hz signals and the cardiac related rhythm on spinally projecting RVLM and caudal medullary raphe neurons rather than on antecedent interneurons in these nuclei ie. that each neuron receives input from both networks generating the 10 Hz rhythm *and* the cardiac related rhythm, although this does not occur for 100% of neurons (Barman and Gebber, 1997). Their group has used bispectral analysis of SNA to further examine the hypothesis that the 10 Hz rhythm and the cardiac related rhythm represent separate generating processes (Gebber *et al*., 1996). This method investigates relationships among different frequencies in the same signal and allows one to determine the degree of coupling between the various frequencies. Standard spectral analysis techniques are based on the assumption that the various frequency components of the signal are independent of each other and a strong degree of coupling between two rhythms may produce an artifact of a new rhythm. In urethane anesthetized cats they observed 64% of the coherence between the cardiac related rhythm and the 10 Hz rhythm could be accounted for by the existence of independent generators of the two rhythms (Gebber *et al*., 1996). They also saw some degree of entrainment between the two rhythms, which they suggested was below the level of generation of these rhythms. They have used the technique of partial coherence analysis where the portion of coherence between the activity of two nerves which can be attributed to a third signal is removed (Gebber *et al*., 1994b). In this way the effect of baroreceptor influences over SNA can be mathematically subtracted. The advantage of this method is that it allows one to test for common sources of input to different signals. By simultaneously recording SNA from cardiac, splenic and renal nerves they found partialization reduced, but did not eliminate the peak at 10 Hz. They suggest this indicates the central sources of the 10 Hz rhythm are not identical in all nerves (Gebber *et al*., 1994b). In fitting with their results they propose a network system for the generation of SNA where the 10 Hz rhythm is not the product of a single pacemaker group of neurons but rather the emergent property of a network of neurons. Subsequently they identified the LTF neurons that may be involved in the control of the 10 Hz rhythm as the strength of its rhythm could be reduced by chemical inactivation (Barman and Gebber, 1993) (Fig. 12). Interestingly this inactivation did not change the power of frequencies < 6 Hz. Such reductions were also observed by muscimol microinjection into the RVLM or raphe (Zhong *et al*., 1993a) but unlike these neurons, the LTF neurons did not have activity correlated to the 10 Hz rhythm in SNA (Barman and Gebber, 1993). They attribute this to the LTF playing a permissive role in governing the 10 Hz rhythm. Discharges of the caudal ventrolateral medullary neurons also have activity which is correlated to the 10 Hz rhythm but not the cardiac related rhythm (Barman *et al*., 1994b) and these neurons can project to the raphe (Barman *et al*., 1995b).

It appears that while there are many reports on the relationship between the 10 Hz rhythm and various cell groups (Table 1), to date a description of how all these connections fit together to produce the 10 Hz rhythm has not been attempted. To some extent this is understandable since the full range of cell groups involved in this rhythm are unlikely to

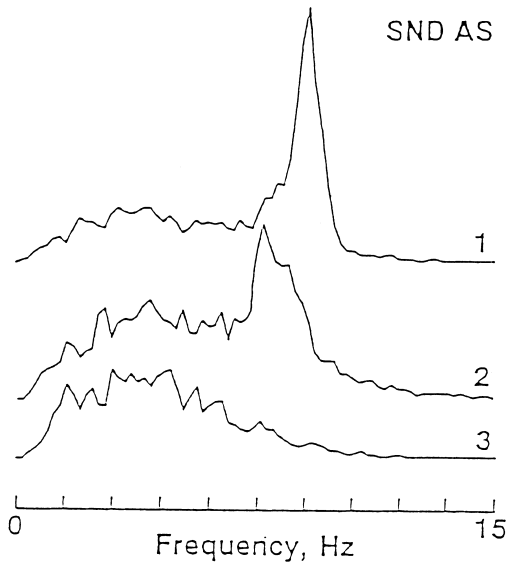

Fig. 12. Effects of muscimol microinjection into the medullary LTF on the power spectrum of cardiac SNA in two baroreceptor-denervated decerebrate cats. Traces 1–3 are auto-spectra of SNA before injection, after injection into the left side of the medulla, and then after injection into the right side of the medulla, respectively. Adapted from Barman and Gebber (1993).

have been identified and there is little information on how the 10 Hz rhythm is altered by a range of physiological stimuli. The other difficulty is that there has been some criticism of the method of defining neurons as part of the system generating certain frequencies of SNA, on the basis of correlation as performed by Barman, Gebber and colleagues (Barman and Gebber, 1992; Barman and Gebber, 1993; Barman *et al.*, 1994a). In particular that this technique tells one little about the function or role of the neurons in the generation of the various rhythms in SNA (Sun, 1995). Indeed, a correlated discharge between two signals provides only evidence that the two tend to discharge at a similar frequency and cannot establish a cause and effect. Thus a neuron may have some activity in the 2–12 Hz frequency band which will probably show some degree of correlation to the 2–6 Hz and 10 Hz rhythm, but this neuron is not necessarily involved in the production of SNA unless proven by other means. The other limitation is that inhibition of certain neurons by chemical, lesion or mid brain transection (Barman and Gebber, 1993; Zhong *et al.*, 1992, 1993a), while able to alter the occurrence of the 10 Hz rhythm, does not necessarily mean that they are involved in the generation of sympathetic discharges but rather may mean that they can influence SNA under some stimuli. This is relevant since the occurrence of the 10 Hz rhythm is altered by stimuli such as asphyxia (Green and Heffron, 1967) or baroreceptor denervation (Malpas and Ninomiya, 1992c). McAllen and May (1996) have further criticized the correlation technique to show connection between neurons. They identified that the correlation method shows both false–negative and false–positive results as commonly as it shows correct ones. Further they suggest that correlation gives information about a neuron's inputs not its outputs, and thus does not prove that one neuron is connected and driving the next but rather that they share some common input.

Until recently, previous studies had almost exclusively proposed the origin of the 10 Hz rhythm to be located in the brainstem (Barman and Gebber, 1997; Gebber *et al.*, 1994b, 1995a) because most of the spontaneous activity disappears after spinal cord transection (Adrell *et al.*, 1982; Zhong *et al.*, 1991). However, an alternative view suggests the spinal cord is the key factor in the occurrence of this rhythm. Partly this stems from the observation that a similar 10 Hz periodicity can be found in the sympathetic discharge of spinal animals when excitability is enhanced (e.g. by asphyxia) (Gootman and Cohen, 1981; McCall and Gebber, 1975). Additionally, Kubota *et al.* (1995) noted that the 10 Hz rhythm could also be observed in anesthetized rabbits and rats. Since the activity of neurons within medulla have a cardiac or respiratory related activity that is species dependent, they suggested that a similar frequency for the 10 Hz rhythm across species indicates that the generating mechanism is separate from the medulla. In support of this they showed that a 10 Hz rhythm could simply be evoked by electrical stimulation of the cervical cord in spinal animals. They went on to explore the hypothesis that the intrinsic properties of spinal preganglionic neurons constitute all the necessary machinery to explain this rhythm, which is automatically gener-

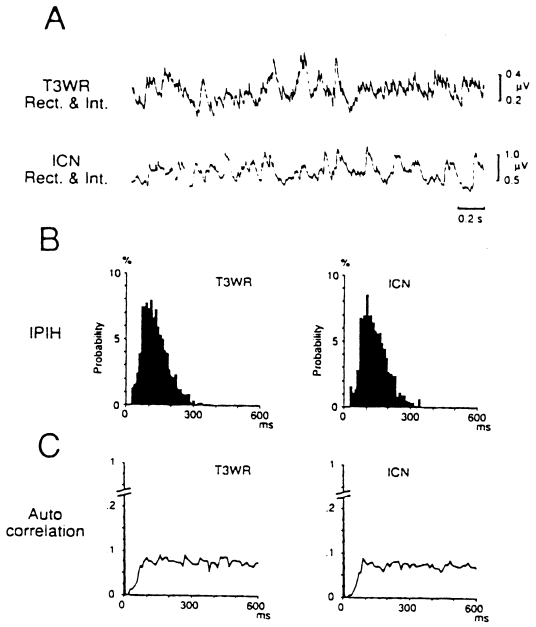

Fig. 13. Activity of the third thoracic segment (T3WR) and the inferior cardiac sympathetic nerve (ICN) evoked by intrathecal injection of NMDA (5 mM) in a spinal cat. (A) Rectified and integrated activities (Rect. and Int.) of the T3WR and the ICN (lower). (B) Interburst probability histograms (IPIH) of the T3WR and ICN. (C) Autocorrelograms of activity peaks of the T3WR and the ICN. After Ootsuka *et al.* (1995).

ated by those spinal neurons when their incoming level of excitatory drive is sufficiently high and sustained (Ootsuka *et al*., 1995). Either electrical stimulation of the dorsolateral funiculus using pulses of 100 Hz or chemical stimulation with intrathecal NMDA to the spinal cord evoked SNA discharges whose distribution had a mode of 100 msec (10 Hz) (Fig. 13).

In summary, the origin of the 10 Hz rhythm may have foundations both within the brain stem and spinal cord and its control is likely to be separate from that producing the cardiac related rhythm. However, it remains to be established that the rhythm is more than a quasi-phenomenon, that is, a product of the experimental conditions and the intrinsic firing properties of the nerves.

12. LOW FREQUENCY RHYTHMS BELOW THE RESPIRATORY RATE

Recently it has become clear that SNA displays oscillations at a number of frequencies below the respiratory rate. Of direct relevance to cardiovascular physiologists is the possibility that these rhythms may directly influence blood flow in various organs to vary at similar rhythms. The magnitude of frequencies in haemodynamic parameters such as heart rate and blood pressure in humans are now being used to estimate changes in sympathetic activity. This is of particular clinical interest since the diagnosis, therapy and prognosis of many cardiovascular diseases may rely on the balance between sympathetic and parasympathetic nervous systems (Zucker *et al*., 1995). The hypothesis has been that there are two slow frequencies in heart rate and blood pressure, one associated with respiration and proposed to reflect vagal tone (Akselrod *et al*., 1981) (this should not be confused with the respiratory oscillation in SNA), and a frequency centred at 0.1 Hz in the human, reflecting the level of sympathetic tone over these variables (Malliani *et al*., 1994). Many researchers have measured these frequencies in heart rate and calculated an index of sympatho-vagal balance defined as the ratio of the two spectral powers in each frequency band. Subsequently there have been numerous papers reporting changes in sympatho-vagal balance in such varied conditions as anesthesia (Introna *et al*., 1995), sleep (Baharav *et al*., 1995) and the menstrual cycle (Sato *et al*., 1995). A mounting number of studies indicate that this hypothesis is flawed on several points (Stauss and Persson, 1995). In general the problem is in identifying a change in the strength of oscillations in heart rate as solely due to changes in SNA if SNA is also not directly measured. DeBoer *et al*. (1987) proposed that baroreflex dependent mechanisms can account for much of the oscillation in heart rate and blood pressure. This has been supported by a study in humans where stimulation of carotid baroreceptors by neck suction at two frequencies (0.1 and 0.2 Hz) could induce a low frequency oscillation in heart rate or blood pressure only if baroreflex sensitivity was normal, and that low baroreflex sensitivity was associated with reduced variability at this low frequency (Sleight *et al*., 1995). They suggested that it is too simplistic to use power spectral analysis of heart rate and blood pressure as a measure of autonomic balance; and that its underlying modulation is more complex than generally believed. Other studies have shown that mechanisms of non-neural origin may influence heart rate at these low frequencies and in particular that the oscillations in heart rate associated with the respiratory cycle, which are normally associated with vagal tone, under some circumstances, cause changes in venous return during manoeuvres such as increased ventilation or exercise and thus affect heart rate (Bernardi *et al*., 1995). Coronary occlusion, which one would normally expect to increase sympathetic tone and thus the strength of oscillations between 0.1 and 0.2 Hz in heart rate, was actually associated with reductions in low frequency heart rate variability (Billman and Houle, 1997). Overall it is suggested that the real advantage in spectral analysis in humans lies in the analysis of interactions between variables such as heart rate and blood pressure and the likelihood that it provides an index of baroreflex function (DiRienzo *et al*., 1996; Parati *et al*., 1995).

With regard to direct recordings of low frequency rhythms in SNA, a distinct rhythm has been identified at 0.3 Hz in the rabbit (Janssen *et al*., 1997) and at 0.4 Hz in the rat (Brown *et al*., 1994). The first observation that should be noted about this rhythm is that it really comprises only a small proportion of the total spectral power of all oscillations in SNA. With regard to renal SNA in conscious rabbits, the power in this band generally comprises no more than 15% of the overall power, with $>50\%$ being contained in frequencies above 3.5 Hz, that is, cardiac and above (Janssen *et al*., 1997). One could argue therefore that this rhythm is of little importance other than of esoteric interest, however, the main difference from the higher frequency cardiac and respiratory related rhythms is that these low frequency oscillations are slow enough to directly induce a rhythm of vasoconstriction and vasodilation in the smooth muscles of the vessels that the nerves innervate (Janssen *et al*., 1997). The end result of these oscillations is that blood pressure also contains a rhythm that is tightly linked to the same frequency (Brown *et al*., 1994). Janssen *et al*. (1997) have shown that oscillations in SNA greater than the respiratory frequency (>1 Hz) do not directly induce oscillations in renal blood flow, but that the slower frequency rhythms between 0.3 and 0.4 Hz can. In support of this Stauss and Kregel (1996) found that electrical stimulation of mesenteric nerves at frequencies >1 Hz did not induce oscillations in mesenteric blood flow, however, those at 0.5 Hz could (Fig. 14). The greatest frequency responses in blood flow were obtained using stimuli between 0.2 and 0.5 Hz. To overcome the artificial nature of electrical stimulation of nerves, in a recent study they performed electrical stimulation of the PVN of the hypothalamus at multiple frequencies whilst recording splanchnic nerve activity and mesenteric blood flow (Stauss *et al*., 1997). Again they found the oscillatory component of the blood flow was negligible beyond stimulation frequencies of 1.0 Hz. These observations imply some filtering characteristic for the vasculature to the various

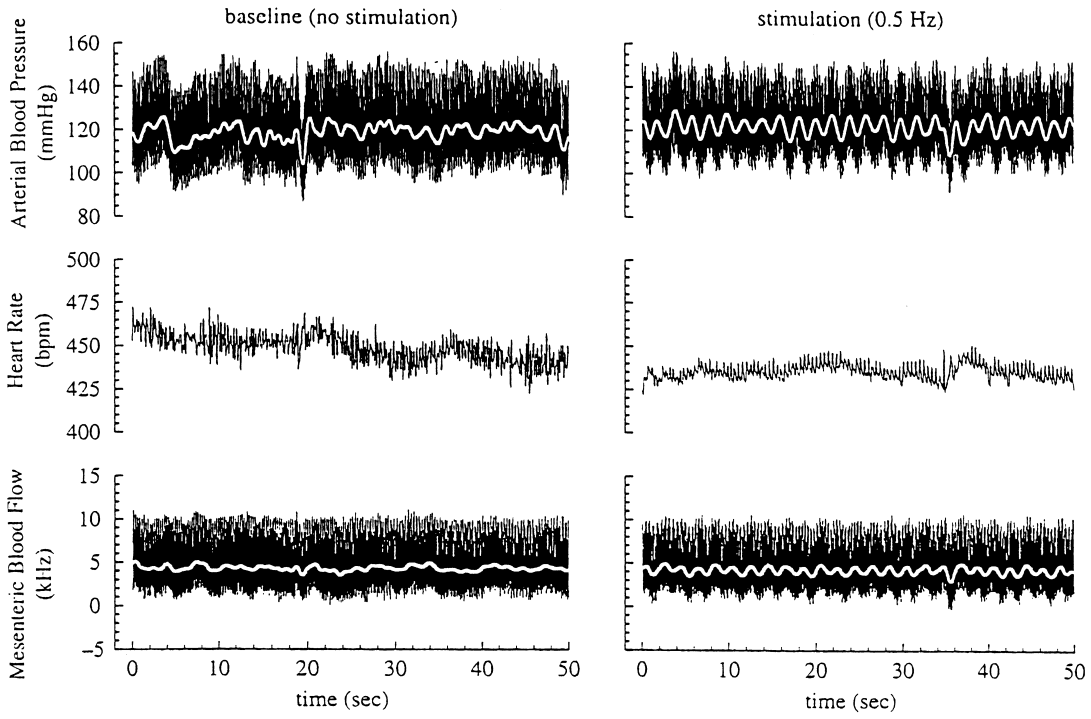

Fig. 14. Original recording of arterial blood pressure (top), heart rate (middle), and mesenteric blood flow velocity (bottom) during baseline conditions (no nerve stimulation) and during stimulation of the left splanchnic nerve with a stimulation frequency of 0.5 Hz. White lines (top and bottom) show mean values. Note the strong oscillatory component in blood pressure and mesenteric blood flow velocity signals during nerve stimulation. Nerve stimulation at higher frequencies saw the ablation of the oscillatory component in blood flow. After Stauss and Kregel (1996).

rhythms in SNA. It had previously been shown that neuroeffector delays at vasculature smooth muscle are sluggish, having time constants of 2–3 sec (Hirst *et al.*, 1992; Hirst and Edwards, 1989; Somlyo and Somlyo, 1990). It was suggested that the higher frequency fluctuations, >1 Hz, are smoothed out by the effector organ response (Janssen *et al.*, 1997). At the present time the precise filter characteristics of the vasculature to SNA oscillations remains to be determined. Similarly, it is unknown whether such vascular responsiveness is uniform throughout the cardiovascular system or if it is differential between target organs. The 'low pass' filtering characteristic of the vasculature to changes in SNA should not be interpreted to indicate that the fast rhythms associated with the cardiac cycle or respiration have no effect on the vasculature, rather it has been proposed that the vasculature integrates these rhythms to maintain a tonic level of vasoconstriction (Janssen *et al.*, 1997).

As described already there are a number of potential pitfalls in assessing changes in spectral power in the low frequency band using variables such as heart rate as an index of sympathetic activity, without actually measuring the nerve activity. A recent study revealed that oscillations in blood flow and arterial pressure could occur at frequencies very close to the rhythm of neural origin but quite distinct from it (Malpas *et al.*, 1998). Haemorrhage was used to activate overall SNA which led initially to an increase in oscillations at 0.3 Hz in renal blood flow of conscious rabbits. The 0.3 Hz rhythm could be directly ascribed to oscillations in SNA as it did not occur in denervated rabbits. However, as blood pressure began to fall, a new rhythm between 0.15 and 0.20 Hz emerged in arterial pressure and renal blood flow in both intact and renal denervated rabbits. Indeed this rhythm also became apparent in SNA, however, its presence in renal blood flow in the renal denervated group indicated that it was likely to be a baroreflex mediated phenomenon, where oscillations in blood pressure baroreflexly caused oscillations in SNA. It was proposed that this rhythm reflects an inherent myogenic oscillation within the vasculature that can be stimulated by the high circulating levels of angiotensin II or norepinephrine that are known to occur in haemorrhage (Korner *et al.*, 1990). Alternatively it may reflect autoregulation. Importantly it was pointed out that the mode of such a rhythm was very close to that of neural origin. Thus when measuring the spectral components of heart rate and blood pressure under different conditions in humans it is not possible to ensure that the increase in power at this frequency (*ca* 0.1 Hz) can be solely ascribed to an increase in SNA.

The origin of the 0.2–0.4 Hz rhythm in SNA is unresolved. Several researchers have suggested it is due to a phase lag in the baroreceptor loop where respiration determines changes in venous return to the heart and consequently blood pressure (DeBoer *et al.*, 1987; Madwed *et al.*, 1989). These changes

stimulate baroreceptors which cause a fast (< 1 sec) vagal response in heart rate and a slow sympathetic withdrawal to the blood vessels. However, rather than buffering the initial change in blood pressure the sympathetic response induces its own oscillation. In support of this hypothesis, perturbing baroreceptor input in humans using neck suction was able to unmask the onset of a slow oscillation in heart rate during a switch from apnea to normal respiration (Bernardi *et al*., 1994). Furthermore, baroreceptor denervation alters the strength of this oscillation's effect on heart rate and blood pressure (Cerutti *et al*., 1994; DiRienzo *et al*., 1996). The alternative possibility is that the low frequency oscillation in SNA is the product of the central network generating the faster frequency rhythms in SNA. At present there is little support for such a hypothesis as studies which have examined activity of cell groups looking for patterns of activation have generally concentrated solely on the origin of the cardiac related or 10 Hz frequencies (Barman and Gebber, 1992; Zhong *et al*., 1993b).

The physiological function of low frequency oscillations in SNA is not clear. One possibility is that organ perfusion is improved by inducing oscillations in blood flow compared to when blood flow remains constant. Another possibility is that in innervated organs, where different functions are controlled by the nerves, these functions may have their responses frequency coded. For example, the renal nerves innervate all sites along the nephron including renin secreting cells of the juxtaglomerular apparatus (Barajas *et al*., 1992; Luff *et al*., 1992). It has been suggested that the frequency response characteristics are different between renal vascular smooth muscle cells and other innervated sites allowing differential control of specific renal functions (Janssen *et al*., 1997).

13. ULTRALOW FREQUENCIES

Unfortunately there have been no long term direct measurements of SNA in conscious animals to ascertain whether sympathetic rhythms exist at frequencies with periods of minutes or even hours. The difficulty lies in maintaining these recordings in an unrestrained environment (i.e.. home cage conditions). Yet there is a real need to establish the role of SNA in the long term regulation of blood flow and thus arterial pressure. It remains to be established whether chronic increases in SNA can be the initiator of sustained increases in arterial pressure. The principal argument against such a response is that arterial baroreceptors adapt to sustained changes in arterial pressure (Andersen and Yang, 1989; Korner, 1989). In addition, denervation of baroreceptors has minimal to no effect on basal levels of arterial pressure chronically (Norman *et al*., 1981; Osborn and England, 1990; Saito *et al*., 1986). This argument assumes, however, that baroreceptors provide the primary chronic feedback signal to the central nervous system. An alternate model has been proposed in which circulating hormones, primarily arginine vasopressin and angiotensin II, provide a long-term afferent signal to the central nervous system via binding to specific receptors in central sites lacking a blood–brain barrier (circumventricular organs) (Brooks and Osborn, 1995). Where the release of the hormones and the sympathetic response to alterations in their plasma concentrations is non-adaptive but may be gated by baroreceptor input. Whether these changes induce oscillations in SNA in the order of ultralow frequencies is unknown. However, the difficulty in performing such studies will be in establishing the cause and effect origin of the rhythms and in proving the oscillations in SNA are not simply the result of some feedback mechanism.

14. HUMAN SYMPATHETIC NERVE ACTIVITY

Since the technique was developed in the 1960s to record SNA in humans, it has uncovered a wealth of information. In general, the properties of SNA observed in animals; such as the synchronous nature of discharges and their timing to the cardiac cycle, are similar in humans, suggesting some commonality between the mechanisms generating SNA across species. Importantly in the case of muscle SNA, it shows strong baroreceptor modulation (Sundlöf and Wallin, 1978). Recent work by Kocsis *et al*. (1997) using partial coherence analysis indicates that while baroreceptor inputs are responsible for much of the strong correlation between SNA to the legs or arms (Wallin *et al*., 1992a), the remaining activity still contains a degree of coherence which cannot be accounted for by baroreceptors. This suggests some degree of coupling between the central networks responsible for cardiac related frequencies targeted to different limbs in the human.

Muscle SNA also displays a respiratory modulation with the greatest activity during late expiration and the first half of inspiration and minimal activity after the peak of inspiration, after correcting for delays within the baroreflex loop (Macefield and Wallin, 1995). By anaesthetizing and artificially ventilating humans it is possible to remove the variation in blood pressure that occurs with breathing. Under this condition a respiratory modulation of sympathetic activity still occurs (Fig. 15). Thus peripheral afferent inputs are not solely responsible for the respiratory oscillation in SNA. This supports observations in animals of an inherent link between mechanisms generating SNA and brainstem respiratory neurons (Pilowsky, 1995). Muscle SNA also displays a low frequency oscillation between 0.05 and 0.15 Hz (Ando *et al*., 1997a), although whether this is a product of baroreflex modulation or central mechanisms is unknown.

The resting level of SNA within subjects appears relatively stable over time (Fagius and Wallin, 1993). This raises the prospect that this value provides a reliable index of sympathetic tone that can be compared across different subject groups. This technique has been applied during a range of stimuli to directly demonstrate, often for the first time, the acute and chronic changes that occur in SNA. Mental stress (Hjemdahl *et al*., 1989), obstructive sleep apnea (Carlson *et al*., 1993) and heart failure

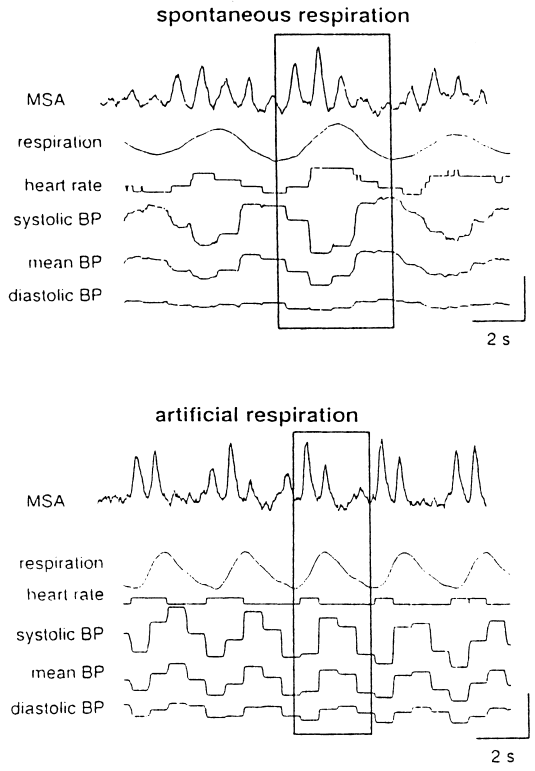

Fig. 15. Averaged records of the respiratory variations in muscle SNA, heart rate and arterial pressure in one subject during spontaneous respiration (top). Inspiration is upwards. Bottom panel shows the same subject during artificial ventilation with intermittent positive pressure. The SNA trace was advanced in time by an amount estimated to correspond to the baroreflex delay (1.14–1.44 sec). In this condition one can note abolished increases in heart rate and reversed variations in arterial pressures during ventilation but preserved phase relationship between respiration and SNA. Vertical calibrations; heart rate = 2 bpm for artificial respiration, 10 bpm for spontaneous respiration, blood pressure = 3 mmHg, muscle SNA was uncalibrated. Adapted from Macefield and Wallin (1995).

(Ando *et al.*, 1997a,b), have been associated with increased SNA levels. Furthermore, in mild hypertension there is some evidence that muscle SNA is also increased (Floras and Hara, 1993). Interestingly the level of muscle SNA shows no relationship to the resting blood pressure level in normotensive subjects. It appears unlikely that this is due to regional differences in the levels of SNA as activity from muscle and renal or cardiac beds is similar when noradrenaline spillover to the kidney and muscle SNA are correlated (Wallin *et al.*, 1992b). Rather, it has been suggested that a balance exists between resting levels of muscle SNA and nitric oxide, and that a stronger level of SNA is buffered by the higher release of the nitric oxide. A positive correlation between muscle SNA and plasma nitrate levels has been identified (Elam *et al.*, 1997). They also hypothesized that altered coupling between sympathetic nerve traffic and nitric oxide release may cause abnormal peripheral resistance, for example, in hypertension.

For technical reasons muscle SNA is normally recorded as multiunit discharges, however, the discharge behaviour of single vasoconstrictor, sudomotor and piloerector nerves has been analysed (Macefield *et al.*, 1994). Their functional classification of the nerves was based on their correlation to the cardiac cycle and changes in arterial pressure where skin vasoconstrictor display no, sudomotor weak, and muscle vasoconstrictor units strong, cardiac rhythmicity. Muscle sympathetic nerves generally discharge only once per sympathetic burst (68% of bursts had only one spike) but multiple firings per burst can occur (Macefield *et al.*, 1994). The mean firing rate for units that fired two or more spikes per burst was 18.8 Hz, however, the distribution was very skewed with the median value at 1.03 Hz. This indicates that the bursts seen in rats, rabbits and cats are unlikely to reflect the same nerves firing more than once in the same burst, as average discharge rates are generally between 1–6 Hz (Malpas and Ninomiya, 1992c). Single discharges in muscle SNA occurred only during the falling phase of arterial pressure, that is, when baroreceptor mediated inhibition of SNA is reduced. Furthermore, Macefield and colleagues noted an increase in the cardiac interval was associated with a higher firing probability, which they reasoned was because in longer cardiac cycles the arterial pressure tends to fall to lower values and thus lessens baroreceptor inhibition. Clearly there is room for confusion between altered baroreceptor input with changes in heart rate and altered input due to a fall in arterial pressure. The two may be quite separate in their effects on sympathetic discharges (see above).

Analysis of the effector organ responses has shown that the skin SNA has both sudomotor and cutaneous vasoconstrictor impulses (Macefield and Wallin, 1996). However, skin SNA is irregular with no relation to the cardiac cycle and is practically absent in relaxed subjects in a thermoneutral environment, although emotional agitation increases the activity. The overall activity is decreased by increases in temperature although there is also evidence of cutaneous vasodilator impulses.

15. ROLE OF THE SPINAL CORD AND GANGLIA IN REGULATING SNA

The dominant theory with regard to the generation and control of SNA rhythmicity places its origin firmly at a supraspinal level (Gebber, 1990). The role of the spinal cord has generally been relegated to an integrative relay point for impulses before travelling to the ganglion. Recent evidence, however, suggests that this has been a hasty conclusion and that under some conditions the isolated spinal cord may be capable of generating or regulating synchronized sympathetic discharges. With regard to overall SNA, most authors report SNA is generally reduced to near noise levels after high spinal cord transection in the cat (Adrell *et al.*, 1982; Gootman and Cohen, 1981; Qu *et al.*, 1988) although in the rat, decreases (Schramm and Choronboy, 1982; Taylor and Schramm, 1987), no change (Meckler and Weaver,

1985; Qu *et al*., 1988) and even increases (Osborn *et al*., 1987; Taylor and Schramm, 1987) have been reported. The one exception in the cat is sympathetic discharges in the splenic and mesenteric nerves, which show minimal change after transection (Meckler and Weaver, 1985). This suggests an unequal dependence on tonic supraspinal inputs for ongoing activity. However, while afferent stimulation can induce changes in SNA, in part mediated by a spinal reflex (Offner *et al*., 1992), these inputs do not seem capable of formulating synchronized bursts of SNA as after spinal transection overall SNA in the greater splanchnic nerve of cats was maintained but there was clear evidence that this was no longer rhythmical (Qu *et al*., 1988). It has been argued that the remaining SNA is likely to result from surgical manipulation and consequent stimulation of afferent pathways (Trostel and Osborn, 1992, 1994).

Allen *et al*. (1993) have reported controversial evidence that under some conditions the isolated spinal cord can generate SNA with a rhythm between 2 and 8 Hz. Their approach in rats was to first remove descending supraspinal inputs to spinal neurons using intrathecal kynurenic acid (a broad spectrum antagonist at NMDA and AMPA/kainate receptors) and then powerfully stimulate spinal neurons with intrathecal kainic acid (a broad spectrum excitatory amino acid agonist). Initially the kynurenic acid decreased overall SNA, and in particular activity in the 2–8 Hz range. Kainic acid was able to restore overall SNA levels to control levels (Fig. 16). Importantly, there was a return of some rhythmicity to the signal in the 2–8 Hz band, although it did not reach the levels prior to kynurenic acid. These observations therefore question the hypothesis that a patterned input from the medulla to the spinal cord is essential for the production of rhythms in SNA. This evidence should not be misconstrued to indicate that the spinal cord generates the rhythmicity of SNA under normal conditions but rather that it contains the necessary machinery to generate such activity if stimulated.

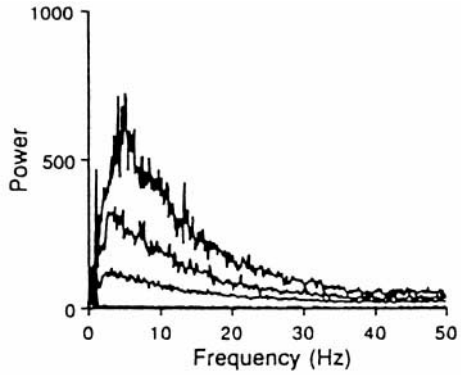

Fig. 16. Power density spectra from one anesthetized rat at three incremental levels of SNA produced at various times after intrathecal injection of kainic acid. Total power in power spectra was initially reduced to 4% of control level by intrathecal injection of kynurenic acid (level with the *x*-axis) and then subsequently raised to 48, 107 and 200% of control level by kainic acid injection. After Allen *et al*. (1993).

Several authors have described the possibility that faster frequency rhythms (>8 Hz) seen in SNA may be the result of spinal mechanisms (Kubota *et al*., 1995; Ootsuka *et al*., 1995). Spinal sympathetic neurons have been shown to have long lasting afterdepolarizations as a result of a calcium-activated potassium conductance (Yoshimura *et al*., 1986) and these may act as low pass filters to descending excitatory activities. Sun (1995) raises the possibility that there might be collateral re-excitation and re-current inhibition networks in the spinal cord which enhance or suppress certain frequencies. Work by Logan and colleagues has identified three types of spontaneous rhythmic activity in intracellular recordings from the intermediolateral cell nuclei of the neonate rat thoracolumbar spinal cord slice preparation: burst firing; tonic beating; and membrane oscillations (Spanswick and Logan, 1990). The frequency of firing in tonic beating neurons ranged from 0.1 to 8.8 Hz. They suggested that a population of neurons in the lateral horn of the spinal cord are capable of rhythmic activity with underlying spontaneous pacemaker-like oscillations. Their recent research indicates that this property is due to electrotonic coupling via gap junctions (Logan *et al*., 1996). They suggest that gap junctions have a low pass filtering action which is important for synchronization and generation of rhythmic sympathetic activity. Moreover they have also suggested that the gap junctions may have different filtering characteristics and voltage sensitivities, and that the degree of coupling between them is modulated by a range of neurotransmitters (Logan and Spanswick, 1997; Pickering *et al*., 1994). This may effect the degree of coherence between nerve activities to different end organs. It could be argued that descending drive, no matter how unpatterned provides a basic input to the spinal neurons which act as low pass filters to produce a variety of rhythms and that direct descending inputs from some supraspinal sources may set the filter frequency.

It is possible that under normal conditions, while not regulating the rhythmicity of sympathetic discharges, mechanisms in the spinal cord govern the number of preganglionic neurons activated by each descending stimuli from the brainstem. In support of this theory a number of cell groups have direct projections to the intermediolateral cell column and bypass the RVLM (Strack *et al*., 1989a,b). Furthermore, the spinal cord has been shown to integrate both excitatory and inhibitory signals (Barman and Wurster, 1978). In summary, the role of the spinal cord in generating SNA remains to be defined but its influence is likely to be considerable in regulating the ongoing frequency of SNA. The intrinsic properties of neurons within the spinal cord may be responsible for the 10 Hz rhythm (see above) and direct projections from supraspinal cell groups with known cardiovascular influences suggests that the spinal cord is not a simple relay station.

It is not generally thought that sympathetic ganglia play a role in regulating SNA rhythmicity, however, it is clear the synaptic interactions in the

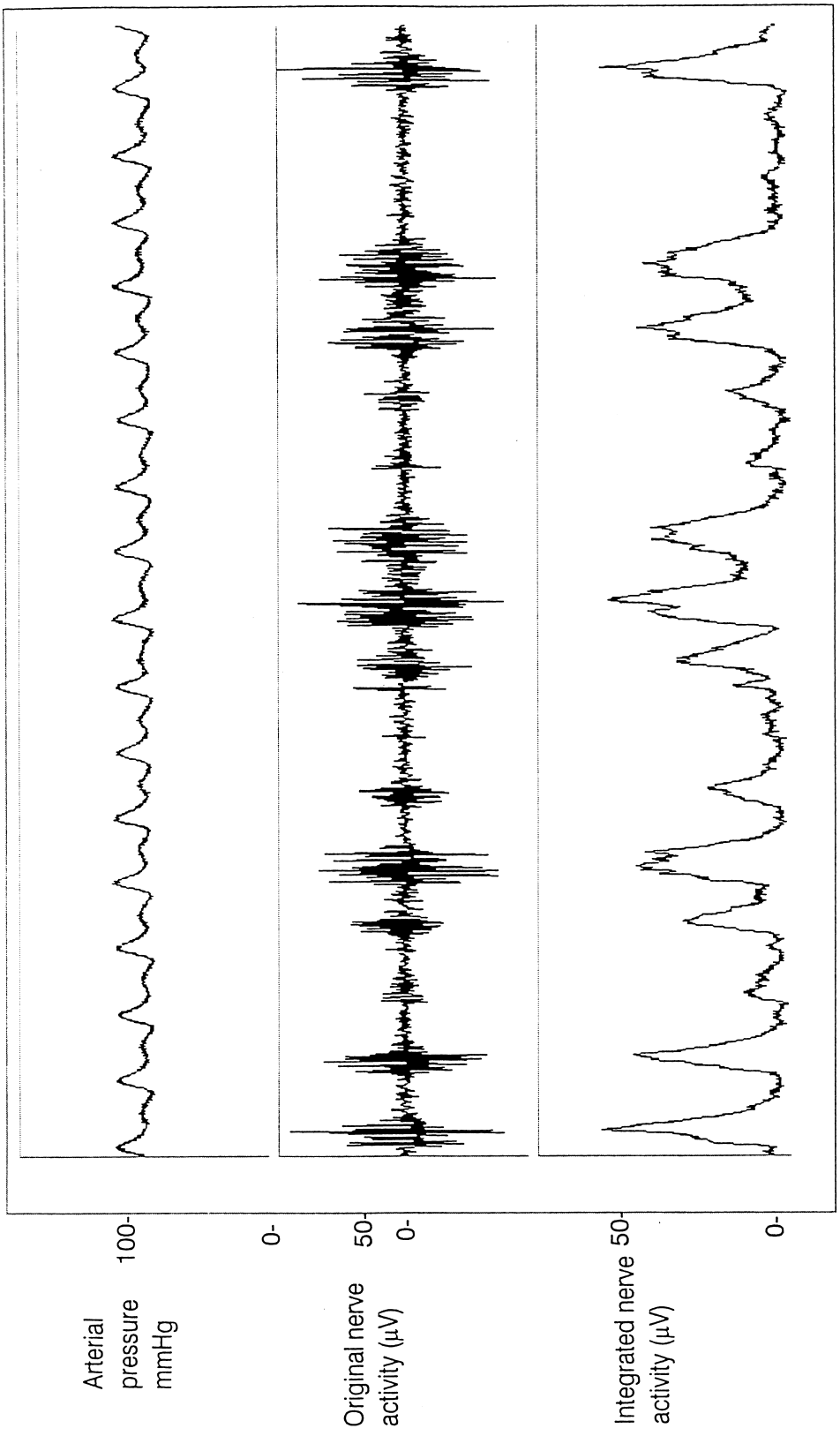

Fig. 17. An original neurogram of renal SNA from one conscious rabbit. The original neurogram was full wave rectified and integrated with a time constant of 20 msec to reflect the discharges as a series of peaks (integrated neurogram). The figure illustrates how most discharges occur with each cardiac cycle and at certain times in the cardiac cycle, but not with every heart beat.

ganglia, different neurotransmitters and neuromodulators can alter transmission of information (Glusman, 1989). Electrical stimulation of sympathetic preganglionic axons elicits a range of excitatory synaptic potentials including fast cholinergic nicotinic, slow cholinergic muscarinic and slow noncholinergic potentials (Karczmar *et al.*, 1986). The predominant view that ganglia have a relay function is supported by recordings from preganglionic neurons which show similar discharge characteristics to postganglionic activity (Bahr *et al.*, 1986; Janig and Szulczyk, 1980). Furthermore Ninomiya *et al.* (1993) have shown that sequential section of preganglionic inputs to the stellate ganglion whilst recording, cardiac SNA, leads to a stepwise reduction in the level of SNA but no change in the frequency of firing.

16. THE AMPLITUDE OF SYMPATHETIC DISCHARGES

While this review has focused on the rhythmical nature of sympathetic discharges it is impossible to do justice to this area without considering that discharges vary not only in their frequency but also in their amplitude. Green and Heffron (1967) were the first to comment on variation in the amplitude of discharges. Their observations raised the possibility that this aspect of SNA may be under separate control from that of the frequency of discharges. They observed activation of right atrial receptors led to a reduction in overall SNA by reducing the amplitude rather than the frequency of discharges. Ninomiya *et al.* (1993) have proposed that the amplitude of discharges reflects alterations in the number of activated nerves within each discharge. It could be argued that a change in the mean amplitude of discharges could reflect the activation of different populations of nerves during stimuli, however, the enormous variation in the amplitude of discharges even between neighbouring bursts (Fig. 17) suggests this is unlikely, under normal conditions at least. In anesthetized cats, baroreceptor activation by increasing blood pressure a small amount with noradrenaline, produced a decrease in the frequency of discharges, but little change in the amplitude of these discharges (Malpas and Ninomiya, 1992a). However, chemoreceptor stimuli produced a selective increase in the amplitude of discharges. This supports the hypothesis that these two components of SNA can be differentially controlled in response to different stimuli (Malpas, 1995). The question arises then as to the location and mechanisms responsible for this control. The possibility that control over the number of recruited nerves is located in the spinal cord was raised above. It should be noted that in the conscious animal this differential control over amplitude and frequency is less distinct, where a full range of baroreceptor activation and deactivation produces a sigmoidal shaped curve in both variables (Malpas *et al.*, 1996). This is not surprising since overall SNA also shows maximum and minimum voltages to changes in blood pressure (Dorward *et al.*, 1985), however, there was some separation of the two variables when animals were also subjected to hypoxia. The blurring of differential control over frequency and amplitude components in the conscious setting may be attributed to the more open nature of the experiments with all afferent reflexes intact. With hindsight, it is apparent that these findings on baroreceptor action in animals had been pre-empted by observations on man: Sundlöf and Wallin (1978) showed that a 10 mmHg variation in diastolic pressure caused a twofold change in discharge amplitude but a fivefold change in the frequency of human muscle sympathetic activity. At that time the implications of these findings for the control of SNA were not pursued, however.

Although it has been well established that the timing of sympathetic discharges is quite closely regulated (Barman and Gebber, 1980; Fussey *et al.*, 1973) there is little information on the regulation of the number of nerves recruited in each synchronized SNA discharge. Variation in the amplitude could exhibit fluctuations which may cluster at several different sizes. However, if one plots the amplitude of a single discharge against the amplitude of the preceding discharge no relationship between neighbouring discharges can be identified, that is, a large discharge was just as likely to be followed by a smaller discharge as it was by a large discharge (Fig. 18). Furthermore, this variability between discharges does not seem to be affected by some stimuli. For example, hypoxia although increasing the mean number of nerves recruited, does not alter the coefficient of variation between discharges (Malpas, 1996). The amplitude of discharges follows a unimodal distribution (Malpas and Ninomiya, 1992d). Further support for the proposal that the amplitude and frequency of discharges are differentially controlled can be found in a plot of the amplitude of sympathetic discharges vs the proceeding interval between discharges (Fig. 19). Although the intervals between discharges conforms either to a burst every heart beat or every two to three heart beats, it made no difference to the amplitude of a discharge whether it was preceded by a long period of no sym-

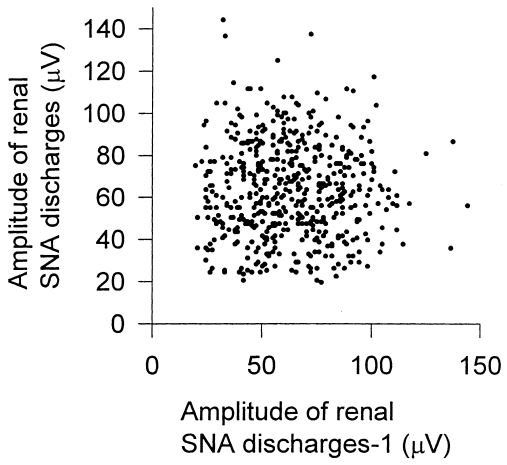

Fig. 18. A Poincaré plot from 500 bursts of renal SNA recorded in a conscious rabbit representing the amplitude of a single sympathetic discharge vs the amplitude of the proceeding sympathetic discharge [data reanalysed from Malpas *et al.* (1996)].

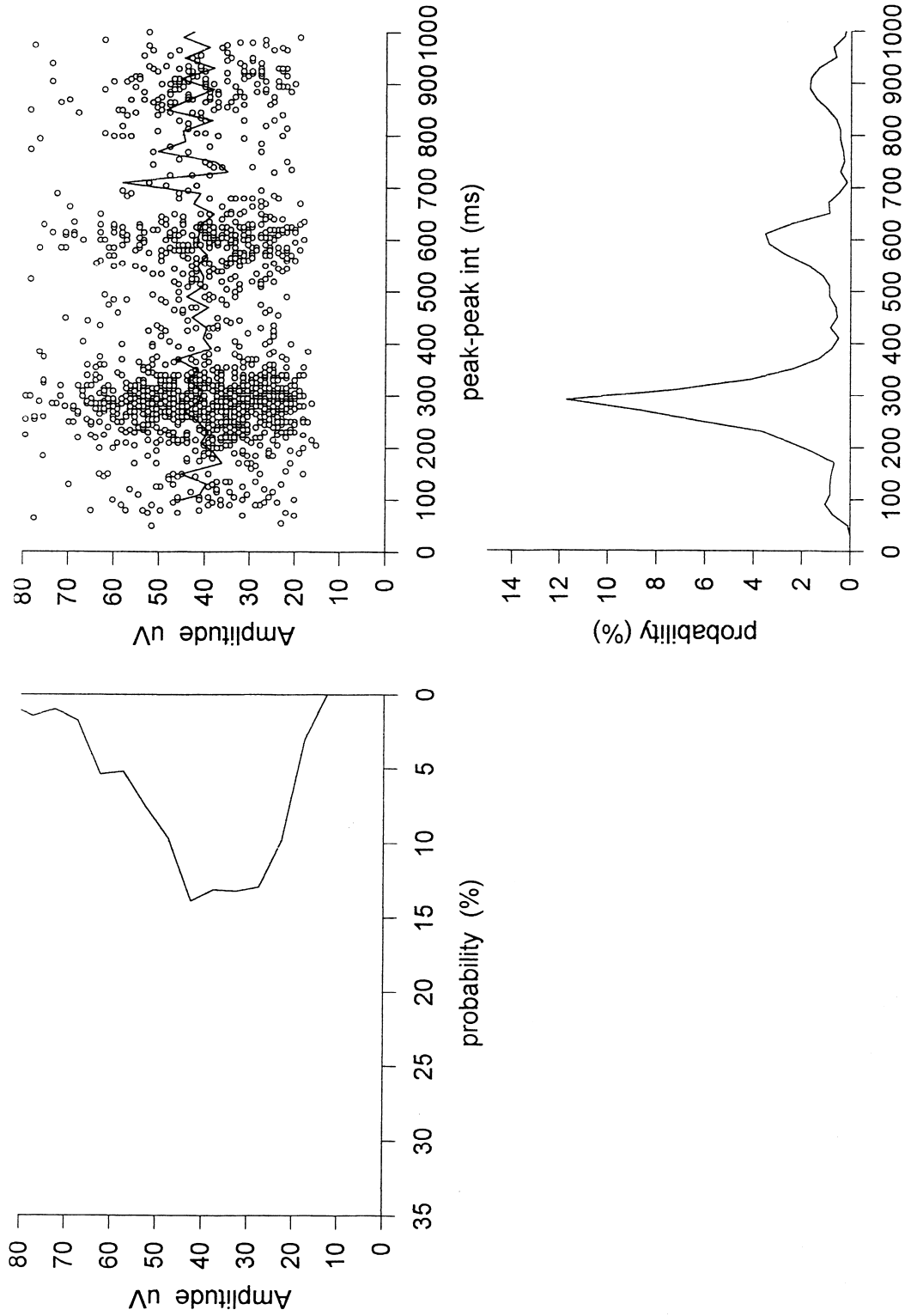

Fig. 19. The individual discharge to discharge data collected from 500 sympathetic discharges in a conscious rabbit [data reanalysed from Malpas *et al.* (1996)]. The scattergram between the amplitude and interval of discharges (peak to peak interval) shows the distinct groupings occurring at each frequency of discharges (top right panel), illustrated in the probability histogram below. However the amplitude of the discharges was not related to the interval between discharges and it made no difference to the amplitude whether the discharge was associated with a long interval between discharges or a short interval as shown by the moving average (solid line) of the amplitude through the scattergram. The top left panel illustrates the probability histogram for the amplitude of discharges.

pathetic activity or many high frequency discharges. Additionally, when discharges occurred that were outside the normal range of discharge intervals, their amplitude was no different from the surrounding population of discharges. This suggests that while the timing of baroreceptor input can affect the probability of a discharge occurring, it does not influence the number of nerves recruited (this timing of the baroreceptor input should not be confused with the level of the input reflecting the blood pressure).

17. RHYTHMICITY OF SYMPATHETIC NERVE ACTIVITY IN DEVELOPMENT

While there have been many studies on neural control of the circulation in neonates, few groups other than Gootman, Sica and colleagues (Gootman *et al*., 1996; Gootman and Sica, 1994; Sica *et al*., 1994; Sica *et al*., 1996) have actually analysed the rhythmicity of sympathetic discharges. In the pig, sympathetic rhythms during development have been identified using spectral analysis. Even at birth sympathetic activity is highly correlated to the blood pressure at the cardiac frequency, providing evidence for baroreceptor modulation (Sica *et al*., 1996). This phenomenon indicates a mechanism for insuring vasomotor tone exists even in newborn animals. Certainly there is good evidence that the integrity of the baroreflex (both sympathetic and heart rate) can be verified in piglets as young as 4 hr old, however, the heart rate component of the reflex appears to differ with postnatal maturation (Cohen *et al*., 1991). Patterns of c-*fos* expression in the brain with hypotension also seem to follow established cardiovascular reflex pathways, further suggesting the neural circuitry is to some extent active at this time (Ruggiero *et al*., 1996). In contrast, spontaneous respiration-related sympathetic discharges were observed only in animals greater than or equal to 20 days old. Such findings suggest a developmental lag in the linkages between respiratory and sympathetic controlling networks. Although in animals <20 days old, hypoxic stimulation and alterations in pulmonary afferents (e.g. lung inflation) could elicit an increase in SNA (Sica *et al*., 1996). A sympathetic rhythm between 8 and 12 Hz appears also present at birth. When the sympathetic activity to different target organs has been compared there appears to be less coherence than later in life. This coherence does not develop until *ca* 19 days of age, which they suggest indicates a degree of postnatal maturation (Gootman *et al*., 1995). Whether this indicates that the independent networks generating SNA to different target organs are not linked until later in postnatal life or that all afferent inputs affecting SNA are not established is unknown.

With regard to the fetus, SNA in the fetal sheep has been shown to play an important role in the regulation of fetal heart rate variability and the heart rate and blood pressure changes during different sleep states (Segar, 1997; Segar *et al*., 1994b). Techniques were developed in 1990 for directly recording renal SNA in the sheep fetus (Smith *et al*., 1990). This has shown the SNA displays synchronized bursts of activity similar to the adult and that it responds to changes in blood pressure. However, the overall level of SNA is much lower in the fetus, rising rapidly at birth (Segar *et al*., 1994a), possibly due to a decrease in environmental temperature at that time (Mazursky *et al*., 1996). There have been no measurements of the rhythmicity of SNA in the fetus, although it is likely a cardiac related pattern of discharges occurs as this was used to ascertain the quality of the recording (Smith *et al*., 1990). Certainly there is evidence that the nerves play a role in the regulation of renal function before birth (Lumbers, 1995) and during birth, as renal denervation blunts the normal increase in plasma renin levels occurring at that time (Smith *et al*., 1991a). However, sympathetic responsiveness to some stimuli is diminished in the fetus as increases in blood volume do not produce a reduction in SNA until after birth (Merrill *et al*., 1994). There is also good evidence to indicate that while the nerves do regulate renal function in the fetus, the effectiveness of changes in SNA is diminished compared to after birth. Electrical stimulation of the renal nerves provokes less reduction in blood flow than after birth (Robillard *et al*., 1987). It has not been identified whether this immaturity is due to differences in the characteristics of the actual nerve activity, the nerve terminals and their contact with smooth muscle, or the ability of the smooth muscle to contract to the ongoing activity.

18. ARE THERE PATHOLOGICAL CHANGES IN SYMPATHETIC RHYTHMICITY?

Overactivity of the sympathetic nervous system has been indicated in a number of pathologies including heart failure (Dibona and Sawin, 1995b), cirrhosis of the liver (Dibona and Sawin, 1995a), and possibly in the initiation and development of some types of hypertension (Anderson *et al*., 1989; Floras and Hara, 1993; Lucini *et al*., 1994). The origin of these increases is beyond the scope of this review, however, altered afferent reflexes (DiBona and Sawin, 1994; Dibona and Sawin, 1995b), and changes in central controlling mechanisms have been implicated (Minson *et al*., 1996).

Interestingly the firing pattern of RVLM neurons was reported to differ between spontaneously hypertensive rats (SHR) and normotensive rats (Chan *et al*., 1991), although it was not defined if these neurons were definitely vasomotor in function. Sun and Guyenet (1986b) have reported that the threshold for inhibition of RVLM neurons by baroreceptors is much higher in established SHR. The problem is in establishing whether these changes are the cause or are the result of adaptation to the increase in blood pressure. One possibility is that chronic changes in particular SNA frequencies may occur in some pathologies. SHR have a different pattern of respiratory modulated SNA (Czyzyk-Krzeska and Trzebski, 1990), characterized by an earlier onset of sympathetic activation during inspiration. These changes were central in origin as they occurred in baroreceptor denervated animals.

Since overall SNA is made up of frequency and amplitude components it must be one or both of these variables that is chronically increased. DiBona and colleagues have examined the frequency characteristics of SNA in congestive heart failure, cirrhosis and nephrotic syndrome (DiBona *et al.*, 1996a). Compared with control rats, all three disease models had increased relative spectral power at the heart rate frequency, indicating an increase in the occurrence of discharges with each heart beat and fewer cardiac cycles with no discharges. The resting frequency of discharges was also increased compared to control rats, in line with an increase in the heart rate in these different models. This raises the potential pitfall in comparing the resting frequency of discharges between groups of animals. Since discharges occur related to the cardiac cycle, a simple sustained increase in the heart rate will lead to an increase in the frequency of sympathetic discharges. Several researchers have recognized this, and also calculate the number of discharges per heartbeat or 100 heartbeats (Gudbjornsdottir *et al.*, 1996; Malpas *et al.*, 1996). Using this index, an increase may suggest the inherent generation of more discharges by the central nervous system or altered baroreceptor input allowing more discharges to 'get through'.

There is an urgent need to establish long term recordings of SNA in conscious animals to test the

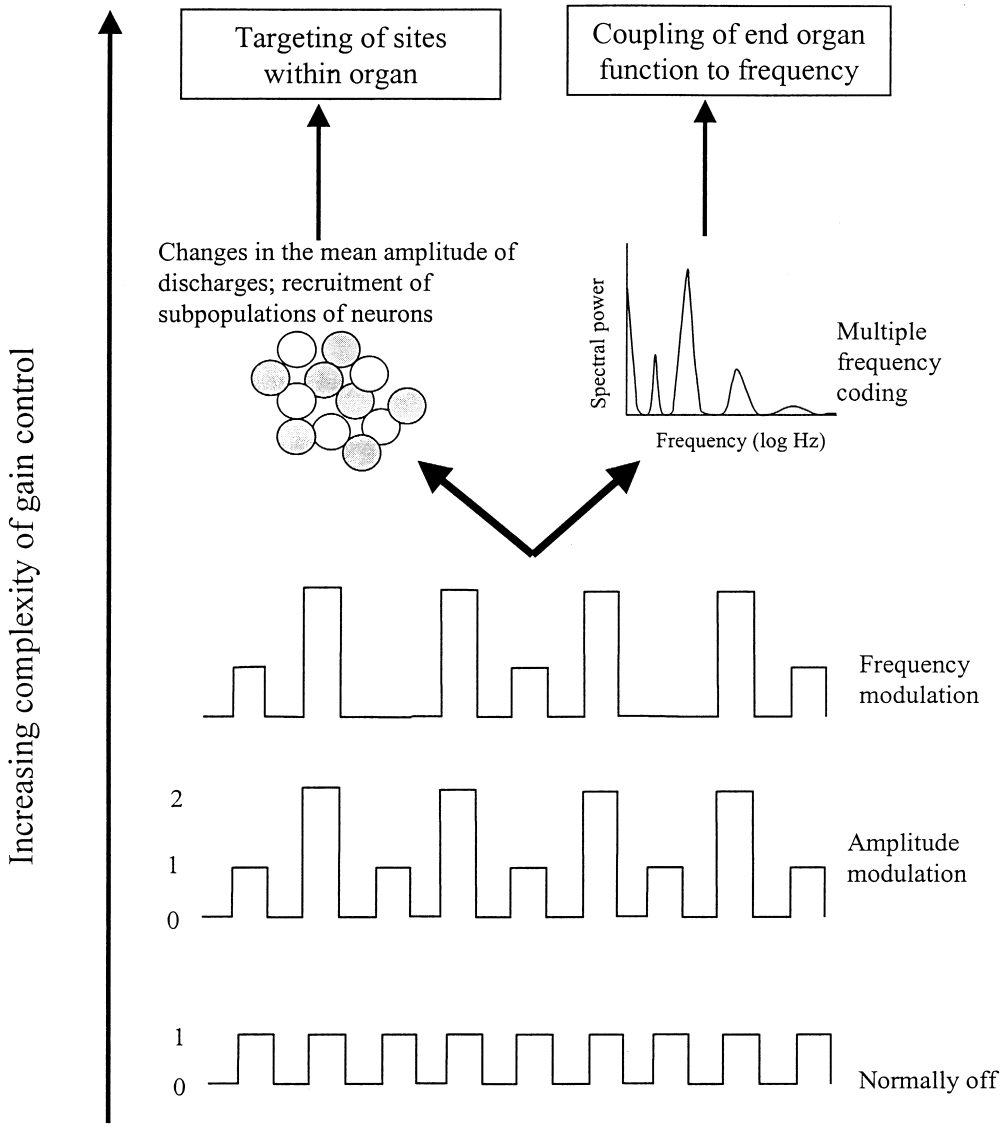

Fig. 20. Schematic illustration of the increasing complexity attainable by amplitude and frequency modulation of SNA. At the first level, with the system normally in the off state, there is only one combination possible. At the second level with amplitude modulation, the number of combinations dramatically increases and allows for a system that is tonically active. Subsequent levels allow for multiple frequency coding either as a function of multiple afferent inputs or due to multiple oscillators. The end result is a system that allows fine control of a variety of end organ functions or variable function at different sites in the organ (recruitment of subpopulations of nerves). All of this processing is performed for each end organ in a differential manner.

hypothesis that a chronic change in the pattern of SNA can be the initiator of a pathological series of events. Given that recordings can be made in conscious rabbits for up to 3 weeks, this species seems the ideal candidate. Such experiments would go some way to defining the role of the sympathetic nervous system in the long term regulation of arterial pressure (Brooks and Osborn, 1995). Although there is no doubt that with regard to short term control of the cardiovascular system the sympathetic nervous system is an important regulator, without studies on its long term relevance the possibility that altered afferent inputs, particularly cardiopulmonary and hormonal systems, could lead to sustained increases in SNA cannot be ignored.

19. CONCLUDING REMARKS

Sympathetic activity can be thought of as an complex output of the central nervous system providing subtle control over end organ function. While it is over 65 years since the first observation that SNA is highly rhythmical, there appears many fundamental questions unanswered. To some extent the area has been held back by the continued measurement of SNA simply as an average voltage per unit of time, such as 1 sec. Such analysis ignores the very dynamic nature of SNA and its division into both frequency and amplitude domains (Fig. 20). While much has been done to identify the central nervous system cell groups involved in producing and regulating SNA (Dampney, 1994a), the way these neurons interact to produce discharges of SNA is unresolved. Evidence seems to favour a network collection of cell groups producing SNA, and anatomical and electrophysiological evidence suggests that there may be separate pathways controlling SNA to the different target organs, although these probably share some common inputs, for example, baroreceptors. It is clear that normal SNA exhibits a large range of frequencies from 10 Hz down to 0.1 Hz and the origin of these rhythms is highly likely to be quite distinct. This raises the possibility that pathologies in which overall SNA levels are chronically increased, reflect changes in the rhythmicity of SNA at certain frequency bands. Once a clearer understanding is found on the origin of these different rhythms it may be possible to delineate more closely the potential central origins of chronic changes in SNA. While SNA clearly regulates blood flow in most organs, there is a paucity of information on how the various rhythms interact with the vasculature. Although the faster rhythms (>1 Hz) may not induce oscillations in blood flow they do provide a level of vasoconstriction, while slower frequency rhythms can induce oscillations. These different frequencies may code for different functional responses within the vasculature.

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