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Homeodynamics versus homeostasis: periodicities superimposed on non-linear dynamic sympathetic tone generated in ventral medulla

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Abstract. Homeodynamics based on theories of complexity and chaos and its impact on mechanisms generating sympathetic activity are presented. Activity in rats cervical, lumbar and renal sympathetic nerves was analyzed. In time domain glutamate stimulation of neurons within medullary periambigual area (PAA) disturbed temporal pattern of respiratory-sympathetic synchronization. Divalent calcium antagonists, Co^{2+} and Mg^{2+}, blockers of synaptic transmission, uncoupled respiratory oscillator and sympathetic activity. PAA neurons act as an interphase between different subsets of respiratory neurons and bulbospinal sympathoexcitatory neurons in rostral ventrolateral medulla (RVLM). In frequency domain sympathetic activity analyzed by FFT algorithm and power density spectra (PDS) exhibited periodicities at the range from 0.4 Hz to 7.5 Hz. Blockers of synaptic transmission microinjected bilaterally into RVLM reduced total power exhibited in PDS to low level of magnitude generated in spinal cord and increased total, yet non-synchronized sympathetic activity and arterial blood pressure. A two component hybrid model of generation of sympathetic activity was proposed: a tone-generating system confined mainly to intrinsic activity of RVLM pacemaker neurons responsible for chaos-like discharges and a second component - neuronal circuits superimposed on tone-generating neurons and shaping the pattern of PDS. Contribution of spinal cord oscillatory mechanism to overall power of sympathetic periodicities was discussed.

Key words: homeodynamics, rat, sympathetic tone, sympathetic rhythm generating mechanisms

INTRODUCTION

Biological sciences, including neuroscience, have reached a time, which Kuhn (1964) defined as scientific revolution characterized by crisis of fundamental paradigms. One side of this scientific revolution represents molecular biology, its spectacular achievements and powerful impact on physiological sciences dominated by overwhelming rise of reductionist way of thinking. This side of scientific revolution is raising again a perennial problem of philosophy of science. Is it possible to deduce the behaviour of a complex living system from the characteristics of its single, interacting parts? Are complex systems with their own specific characteristics deducible from their components? The opposite side of the present scientific revolution, not yet appreciated enough, yet equally important, is a rise of the new sciences of complexity and their impact on biology. Discovery of non-linear dissipative self-organizing structures (Nicolis and Prigogine 1977), synergetics (Haken 1983) and, first of all, birth of non-linear dynamics under general term of deterministic chaos (see Schuster 1984, Ruelle 1991) provided mathematical tools to analyze complex systems containing many interacting single elements. Living systems offer an ideal object for this new science, because they are complex and self-organized in a fashion far from linearity in the classical Newtonian sense. Molecular biology describes living systems mainly on structural and informational levels (codes and messages), leaving aside functional dynamical level, at which plasticity and new patterns are generated. New dynamic approach to complexity offers a hope for the future to overcome rising conflict between the reductionist and holistic approaches to life, the last one assuming nonreducibility of living complex system.

Time came to reexamine one of the classical paradigms of physiology and biology. This paradigm has been a corner stone of physiology since it was formulated by Claude Bernard. He was the first one to express the paradigm in the famous classical sentence written in his last book (1878) published and left behind, like a testimony, at the year of his death: "La fixiti du milieu interieur est la condition de la vie libre, independante" ("The constancy of the internal environment is a precondition of the free and independent life"). His great successor, Walter Cannon, who introduced the concept and term homeostasis, probably the most frequently used word in physiological papers and textbooks, imprinted as a framework of thinking in the minds of students and scholars, wrote in his last, very personal book (1945) also as a kind of testimony published at the year of his death: "Our bodies... are composed of highly unstable material. They are subjected frequently to disturbing conditions. The maintenance of a constant state within them is in itself evidence that agencies are acting or are ready to act to maintain this constancy". Yet no physiological parameter, even in most resting and relaxed situation, is constant. Stasis means literary stagnancy, stillstand or, at the best, a perfect equilibrium. But stillstand, if anything, means rather death, never life.

Fluctuations of physiological parameters *in* steady state conditions, usually ignored by averaging or masked by routine statistical tests, can no longer be dismissed as unimportant biological or stochastic noise, imperfection or error of simple closed-loop feed-back homeostatic control systems. Instabilities and nonequillibrium got a new and meaningful dimension, because they express some of the fundamental features of life as self-organizing and plastic system generating true novelty.

The essence of the present scientific revolution rejecting an old paradigm is recognition that oscillations, fluctuations and non-linear instabilities effectively protect integrity and provide plasticity by generating new behavioral patterns in a living system confronted with external or internal perturbations. "At all levels, be it the level of macroscopic physics, the level of fluctuations or the microscopic level, nonequillibrium is the source of order. Nonequillibrium brings order out of chaos" (Prigogine and Stengers 1984). That is why Yates (1993a, b) proposed recently a new term "homeodynamics" instead of homeostasis. Figure 1 shows the main dif-

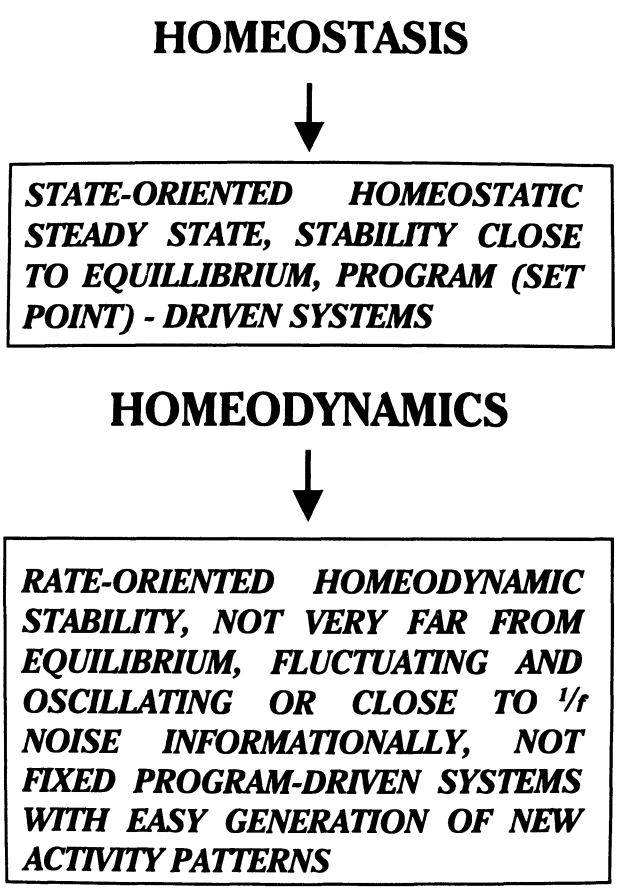

Fig 1. Main characteristics and differences between Cannon's concept of homeostasis and homeodynamics proposed by Yates (1993).

ferences between the new and old concepts. Homeodynamic concept is by no means a finite theory. It has still to overcome a principal theoretical difficulty in order to be fully applicable to living systems. It is based on deterministic chaos theory and on space oriented trajectories in the topological sense. Such trajectories are extremely sensitive to initial conditions. Basins of strange attractors, which in biological terms mean near-equilibrium state, are extremely unstable (for references Schuster 1984). Yet, being non-linear, living systems are able to preserve their integrity and in long term do not deviate far from equilibrium. A way to control chaos in biological systems has to be postulated. To resolve this difficulties new theoretical solutions have been proposed from the biological point of view (Yates 1993a, b) and by mathematicians and engineering scientists as so called OGY method (for references Ditto and Pecora 1993). Perhaps, ways to control chaos that were tested with success in technical sciences are similar to those used by living organisms. So far, however, such possibility remains still a challenge for future cooperation between life scientists, physicists and mathematicians.

In Cannon's homeostasis concept sympathetic nervous system served as a powerful mechanism for survival of the body and for recovery of the "internal milieu" from "fight or flight" disturbances to the homeostatic steady state. Yet, in the so called steady-state resting conditions sympathetic activity is by no means stable. Fluctuations and rhythmicities were discovered just by the first direct, still crude recording of electrical sympathetic activity (Adrian et al. 1932). Respiratory-related and cardiac-related rhythmicity was reported. This classical observation has been confirmed many times since (for references Koepchen et al. 1980). For decades fluctuations in sympathetic activity have not attracted much attention. Their significance is to be recognized now with new dynamic way of thinking in terms of homeodynamics.

In cat, the species most frequently used for studying rhythmicities in the sympathetic activity, maximal sympathetic rhythmic discharge corresponds to inspiratory phase. Strong depression appears always in postinspiratory and early expiratory period (Bainton et al. 1985). However, our studies showed that this pattern of respiratory-sympathetic synchronization in time domain can not be generalized as it is species-specific (Trzebski 1991). In rats the increase in spontaneous sympathetic activity appears rhythmically not in inspiration but in expiration (Fig.2) (Czyżyk et al. 1987, Czyżyk-Krzeska and Trzebski 1990). In humans temporal pattern of respiratory-sympathetic synchronization appears to be situated somehow between these two extremes which characterize cats and rats (Eckberg et al. 1985). We have proposed a hypothesis that temporal pattern of respiratory-related sympathetic rhythm is adjusted to species-specific behavioral habits (Trzebski 1991). In diving animals, like rats or muskrats, expiratory increase in the sympathetic

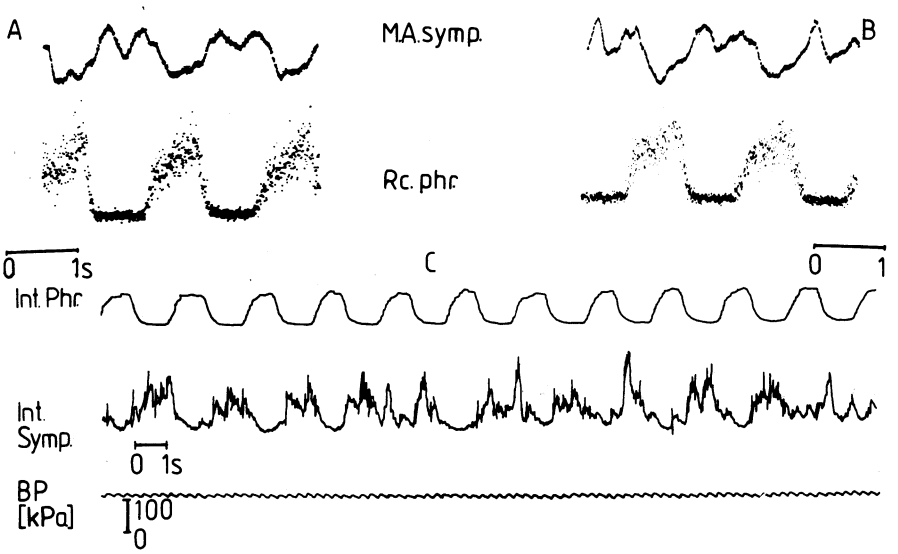

Fig 2. A and B, averaged and integrated spontaneous activity in rat's cervical sympathetic nerve (MA Symp) triggered by the onset of integrated phrenic burst (Rc. phr.) computed at the respective time periods of the continuous original recording shown below (C) of the integrated phrenic nerve activity (Int. Phr.), integrated sympathetic nerve activity (Int Symp.) and arterial blood pressure (BP).

discharge corresponds to the pattern of diving response. It starts in expiration and is characterized by sympathetically mediated vasoconstriction, an important oxygen saving mechanism during submersion.

Neuronal population generating sympathetic activity serves as a major supraspinal common pathway for reflex and central sympathoexcitatory influences and is confined to bulbospinal neurons in rostral ventrolateral medulla (RVLM) (for references Ciriello et al. 1986, McAllen and Dampney 1989, Dampney 1994). First direct evidence that stimulation of ventrolateral medulla produced sympathoexcitatory response was provided already in 1971 (Trzebski et al. 1971, Trzebski et al. 1974).

The purpose of this study was to elucidate the role of neurons within RVLM in rhythmical respiratory-related synchronization of sympathetic activity in time domain, and, furthermore, to test the significance of RVLM neurons for other sympathetic periodicities analyzed in frequency domain in time series. Some of experimental data, published elsewhere (Baradziej and Trzebski 1989, Baradziej and Trzebski 1991, Trzebski and Baradziej 1991, Trzebski and Baradziej 1992), provided experimental material for this study in which a new dynamic model of the central generation of sympathetic activity is proposed. We conclude that two distinct central mechanisms contribute to overall sympathetic activity: rhythms originating in central neuronal circuitries, mainly inhibitory, superimposed on sympathetic tone of a non-linear, apparently chaos-like activity, generated by RVLM pacemaker excitatory bulbospinal neurons.

METHODS

Material and surgical procedures

Experiments were carried out on 42 male Wistar rats anesthetized either with urethane administered intraperitoneally or urethane combined with chloralose (section 1 and 2 of Results) or with Saggatal i.v. (section 3 and 4 of Results). Animals were paralyzed by i.v. administration of pancuronium bromide (Pavulon, Organon Hesse), artificially ventilated with oxygen-enriched room air by respiratory pump, blood pressure being recorded from the femoral artery as described previously (Czyżyk-Krzeska and Trzebski 1990). Monitoring and maintaining the blood gases at proper levels were performed by AVL 95 Automatic Blood Gas System in 0.1 ml blood samples taken from the femoral artery and replaced by the same volume of donor animal's blood. In addition, end tidal CO_2 concentration was monitored by Beckman LB-2 Gas analyzer and maintained at the level of 4% E_TCO_2 by adjustments of frequency of the ventila-

tory pump. In most experiments animals were va*gotomized* and carotid sinus nerve bilaterally cut. Spinal transection was performed carefully and very slowly to avoid excessive bleeding and damage to the spinal cord. The rectal temperature was controlled and maintained at 37°C. Ventral surface of the basooccipital skull was exposed after animals has been placed in supine position. A large window 6 mm wide and 8 mm long was drilled to expose the ventral medulla (Fig.3). Microinjections of 0.5 M glutamate into RVLM (retrofacial nucleus) were performed under binocular control via glass micropipettes using micromanipulator. Pico Pump WP1 microinjector connected to pressure reservoir was used for this purpose according to procedure described in detail previously (Baradziej and Trzebski 1989). Blood pressure rise and sympathoexcitatory response following microinjection of 10-20 nl of glutamate was accepted as a criterion for functional mapping of RVLM sympathoexcitatory neuronal bodies. Synaptic input and synaptic transmission within RVLM neurons was blocked by divalent calcium ion antagonists, $COCl_2$ or $MgCl_2$ (Paton et al. 1991), that were microinjected into RVLM in the 150-300 nl volumes and 12 mM or, occasionally, 4 mM solutions. The injection was performed very slowly in order to avoid damage to the tissue. Saline solution of the same volume and speed of microinjection was used for control. In some experiments 0.5 M kynurenic acid (100 nl), an unspecific glutamate receptor antagonist and 0.01 or 0.5 M sodium kainate, an unspecific, powerful NMDA and non-NMDA glutamate receptor agonist were used either for blocking glutamatergic transmission or, respectively, for the destruction of cell bodies around the site of microinjection. Hexamethonium i.v. was used for blocking synaptic transmission in sympathetic ganglia. The sites of microinjections were confirmed by the standard histological procedure using microinjections of pontamine sky blue into the same sites. For histological verification the brain was slowly perfused with 10% formaline solution under 20 mm Hg at the end of experiment. The brains were then removed and medulla oblongata sectioned on Kryostat Microtom into 60 m transverse sections. The injected structures were identified according to the stereotaxic atlas of Paxinos and Watson (1986).

Nerve recording and stimulation

Details of the sympathetic nerve dissection were published elsewhere (Czyżyk-Krzeska and Trzebski 1990). Efferent activity was recorded in the preganglionic cervical nerve and in the postganglionic filaments of lumbar nerve and/or renal sympathetic nerve. Phrenic nerve activity was recorded in parallel with the sympathetic nerve activity and integrated with a RC circuit (time constant 0.1-0.2 s). Sympathetic nerve activities were amplified and integrated by the method of moving time averaging (bandpass 50 Hz-2 kHz). In some experiments sympathetic nerve activity was integrated with a RC circuit of time constant 0.2 s, fed into a window discriminator which passed discharges surpassing electrical noise level. The level of "zero" biological sympathetic activity was determined by recording from the nerves after the animal had been killed with an overdose of i.v. Nembutal. All data, including arterial blood pressure, were recorded on the Ampex SP 300 or Racall 7 DC tape recorder for off-line analysis, and displayed on the polygraphic ink injector recorder Mingograph 7 (Siemens). Stimulation of the central end of the cut aortic nerve and of the vagal trunk was applied by Digitimer 4030 programmed stimulator with 3-10 pulses of 0.5-1.0 ms duration and 5-30 Hz frequency. Absence of the sympathetic responses to afferent nerve stimulation was accepted as a criterion of effective blocking of synaptic input into and within RVLM neurons (Sun et al. 1988). Details of the procedures were described previously (Trzebski and Baradziej 1992).

Data collection and analysis

In time domain integrated respiratory-related spontaneous sympathetic rhythmicities were averaged over ten to sixteen respiratory cycles with ANOPS 101 averager or Cambridge Electronic Design Ltd 1401 real time computer using as a trigger

a single stimulus delivered from the programmed stimulator Digitimer 4030 at the onset of the integrated phrenic burst. Intervals between phrenic bursts were accepted as the duration of the expiratory phase T_E. Inspiratory period T_I was calculated from the onset of the phrenic discharge to the point of delay of phrenic activity.

In frequency domain, 10 s windows were accepted as time series satisfactory to assume stationarity for computation of power density spectra (PDS). PDS were expressed in normalized amplitudes, the value in each frequency bin representing the fraction of total energy that was presented in PDS. Spike signals were computed by FFT algorithm and processed by Spike-2 Cambridge Electronic Design program. Integrated activity was filtered and sampled at 100 Hz. A slow component was eliminated by low-pass filtering. The spectra obtained for the different data sets were averaged and computed in consecutive time series 10 s each. The bands within individual frequencies were broad, suggesting aggregate rhythm of superimposed activities of individual fibers within nerve filament and/or non-linearity of the spike intervals. For quantification of PDS data, integrated values of PDS curves (surface areas) in three main frequency bands (respiratory, intermediate and cardiac) were computed and compared for statistical analysis.

Respective mean values of each group of nerve spectra were compared and the significance of differences was checked by Student's t test. Respective formula was chosen on the basis of similarity of dispersion curves of different sets of data evaluated by Snedecor's F test. $P<0.05$ was accepted as significant for all tests used. Variability was expressed as means ±SE.

RESULTS

Effects of stimulation of periambigual neurons on the rhythmical respiratory-sympathetic synchronization in time domain

Periambigual area (PAA) was defined as a small region between ventral respiratory group neurons (Schwarzacher et al. 1991) and bulbospinal excitatory sympathetic neurons within RVLM. Contra-

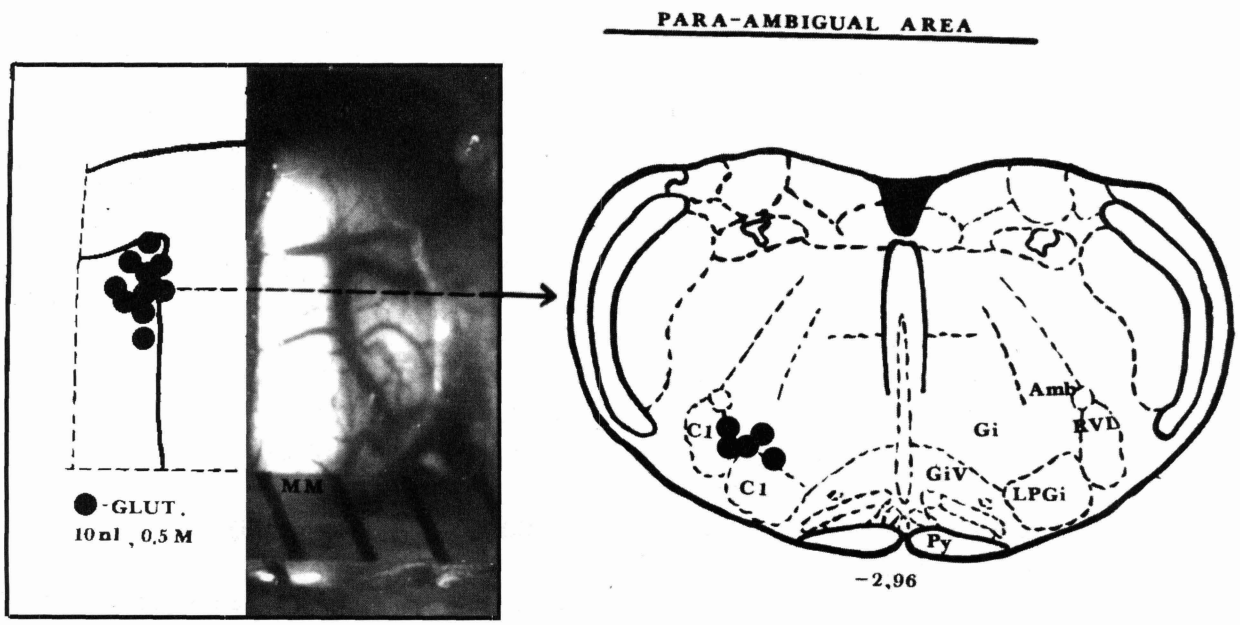

Fig 3. On the left, exposed ventral medulla: on the right, sites within periambigual area (PAA) where the glutamate, $CoCl_2$, $MgCl_2$ and kynurenate microinjections were performed. C_1 marks rostral ventrolateral medullary area (RVLM). Other abbreviations after Paxinos and Watson (1986).

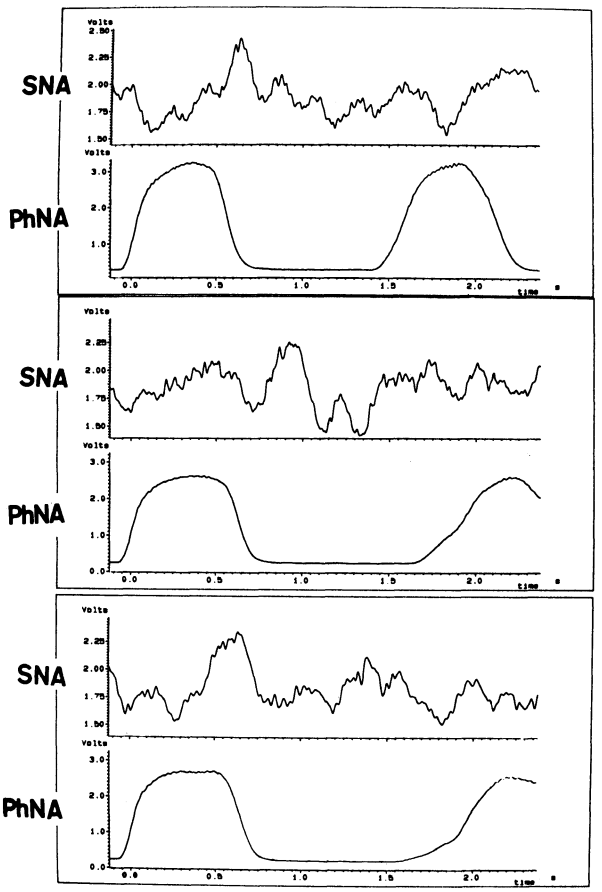

Fig. 4. Top section, averaged integrated lumbar nerve sympathetic activity (SNA) and phrenic nerve activity (PhNA). Middle section, change in the temporal pattern of respiratory- sympathetic synchronization following 10 nl 0.5 M glutamate microinjection into PAA. Bottom section, the pattern after 15 min.

ipsi- or bilateral microinjections of 10-20 nl sodium glutamate into PAA (Fig.3) changed in a variable way the temporal pattern of sympathetic discharge within individual respiratory cycle (Fig.4). The effect depended on the site of microinjection within PAA. Some microinjections (Fig.5) totally reversed the pattern of respiratory sympathetic synchronization, mimicking the pattern which is typical rather for cats (Bainton et al. 1985) or for spontaneously hypertensive rats (SHR) of Aoki-Okamoto strain (Czyżyk-Krzeska and Trzebski 1990). Usually no significant change in the respiratory cycle or in the amplitude of phrenic bursts was observed unless microinjected volumes of glutamate were very large . Effects were reversible as essential pattern of synchronization, sympathetic depression in early inspiration and expiratory-related facilitation, returned after about 15 min since the onset of microinjection (Fig.4).

Effect of $CoCl_2$, $MgCl_2$ and kynurenic acid microinjections into periambigual area on respiratory-sympathetic synchronization in time domain

Bilateral microinjections of divalent calcium ion antagonists Co^{2+} or Mg^{2+} in 12 mM solutions of $CoCl_2$ and $MgCl_2$ almost totally abolished any res-

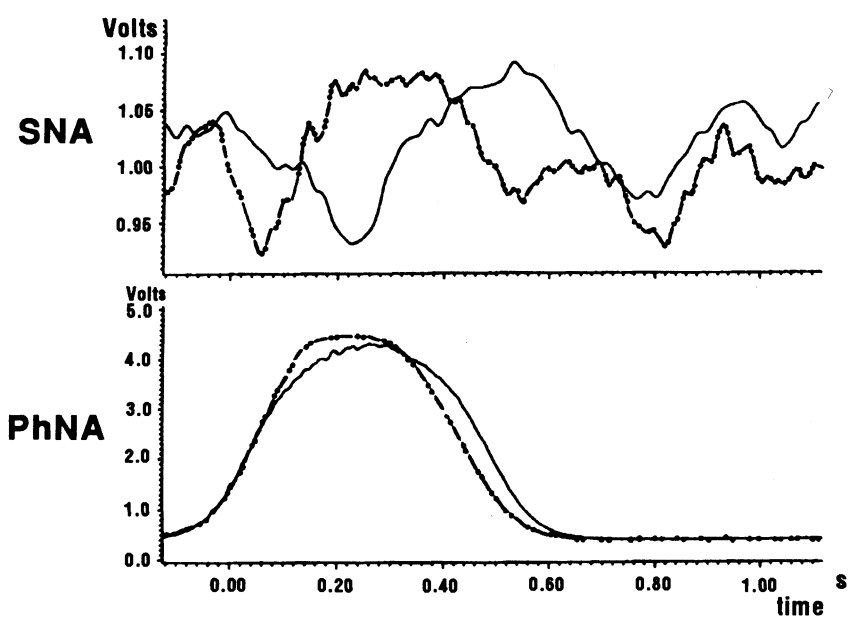

Fig. 5. Two superimposed recordings of the averaged integrated sympathetic lumbar nerve activity (SNA) and phrenic nerve activity (PhNA). Solid line, control pattern of respiratory - sympathetic synchronization characterized by inspiratory-related depression, and expiratory-related excitation of sympathetic discharges. Broken line, inversed temporal pattern produced by 10 nl 0.5 M glutamate microinjected into PAA.

piratory modulation of the sympathetic nerve activity (Fig.6). Within 10-20 min this effect disappeared and could be reproduced again by repeated microinjections into the same PAA region up to several times during each experiment. Neither phrenic nerve discharge amplitude, respiratory frequency or T_I and T_E were significantly influenced by calcium ions antagonists applied into PAA. Therefore, a total dissociation of the respiratory rhythm from on-going sympathetic activity could be accomplished by unspecific blockers of synaptic transmission applied locally in PAA. Kynurenic acid was less effective and usually depressed phrenic nerve bursts after microinjections into PAA (Fig.6).

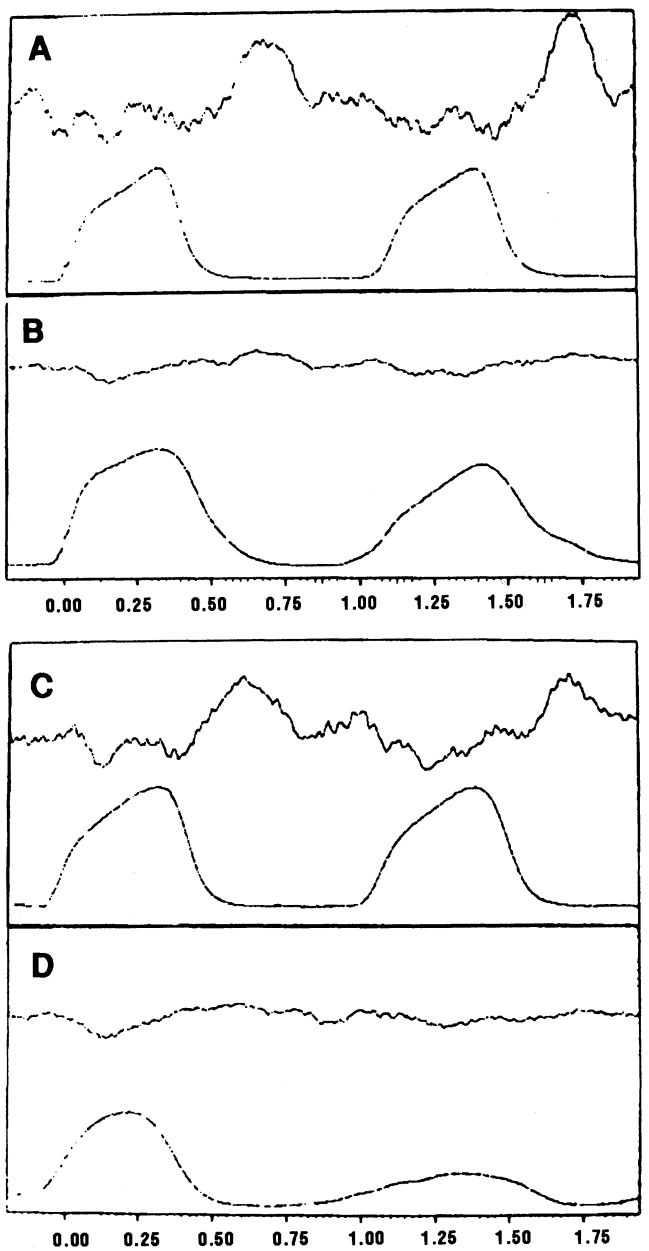

Fig. 6. A, averaged integrated recording of the activity of sympathetic lumbar nerve (top) and phrenic nerve (bottom) in control conditions; B, effect of microinjection of $MgCl_2$ into PAA. Respiratory and respiratory-related sympathetic rhythms entirely uncoupled; C, return to control pattern after 20 min; D, the effect of microinjection of 300 nl 0.5 M kynurenic acid into PAA. Respiratory and respiratory-related sympathetic rhythms are not entirely dissociated and the amplitude of phrenic bursts is reduced.

Effect of blocking the synaptic transmission to RVLM on the sympathetic periodicities analyzed in frequency domain by power density spectral analysis

In this experimental series twenty rats with baro- and chemoreceptors denervated and anesthetized by i.v. Saggatal, were used. Initially 4 mM, usually, however, 12 mM $CoCl_2$ or $MgCl_2$ solutions were microinjected bilaterally into RVLM. Power density spectra (PDS) of sympathetic nerve activity computed in 10 s time series demonstrated that total power was confined within 0.4-7.5 Hz frequencies band. No sympathetic rhythms above 8 Hz were observed. The shape of PDS was characterized by several peaks very variable in individual experiments. Respiratory frequency- -related peaks were of high amplitude and spread over wide band of frequencies. The peak in PDS corresponding to cardiac frequency was low and not sharp in the total band of related frequencies. In a few experiments in which vagal, aortic and cardiac nerves were uncut and baroreceptors remained intact, cardiac rhythm was well exhibited in PDS. In contrast to PDS computed in cats (Gebber et al. 1989), in rats bands corresponding to individual frequencies were broader and the distinction between dominating frequencies in the whole spectrum was less clear. Most of the power was concentrated in the band from 2.5 to 5 Hz at the range intermediate between respiratory related frequency, including its harmonics (0.7 to 2 Hz) and cardiac frequency range (5.5 to 7.5 Hz).

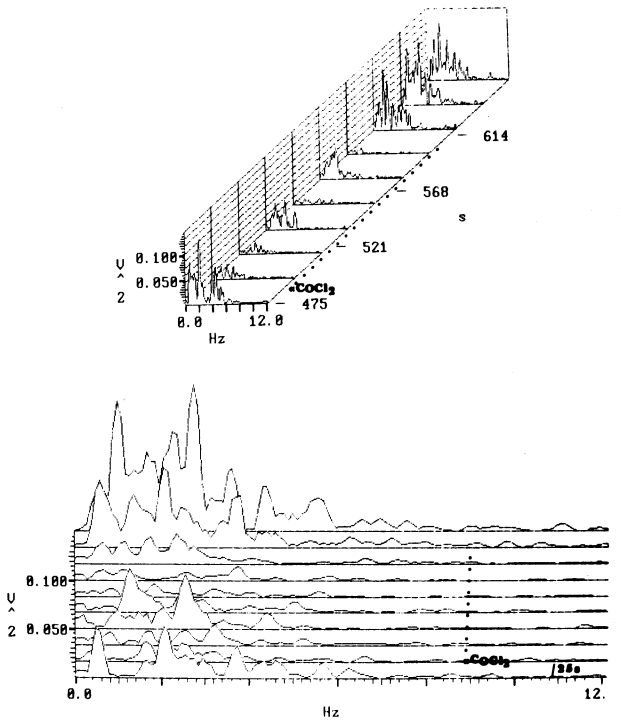

Fig. 7. Normalized power spectra (PDS) computed in consecutive 10 s time series, from the efferent activity in sympathetic cervical nerve (top records) and in lumbar nerve filament (bottom records). Microinjections of 150 nl 12 mM $CoCl_2$ marked on the figure. Transient yet dramatic depression of power in PDS. A rebound effect, oversynchronization, appeared in the lumbar nerve activity (bottom).

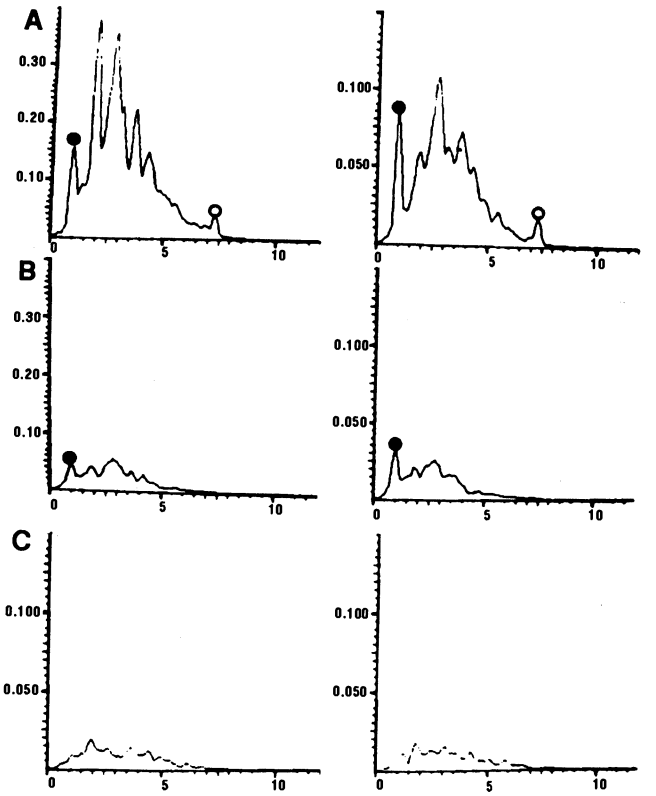

Fig. 8. A, power density spectra computed from the efferent activities of two separate filaments of the sympathetic lumbar nerve. Filled circle, respiratory related frequency peaks, open circle, cardiac frequency related peak; B, power density spectra of the same nerve fibers after bilateral microinjections of $MgCl_2$ solution into RVLM; C, power spectra computed after spinal cord transection in the same rat and from the same nerve filaments activities.

After bilateral microinjection into RVLM of divalent calcium antagonists, the power over all frequencies was dramatically reduced (Fig.7). The effect lasted for 15-20 min and, occasionally, a short lasting rebound appeared (Fig.7). After total recovery, repeated bilateral microinjections of $CoCl_2$ or $MgCl_2$ again reduced the power over all bands of frequencies. In 5 rats spinal cord was transected at the level of C_2-C_3. Spinalization reduced power in PDS to a magnitude comparable to that observed after blocking synaptic transmission to RVLM (Fig.8). Remnant periodicities remained only in the intermediate frequency band (2.5-5 Hz), which represents the fraction of maximal power concentration in PDS of intact animals. Respiratory- and cardiac-related frequencies were absent in spinal animals (Fig.8).

In separate experiments PDS time series were computed from activities recorded in parallel in preganglionic sympathetic cervical trunk fibers and in postganglionic fibers within filaments of dissected lumbar sympathetic nerve. In the rat's cervical trunk only preganglionic sympathetic fibers are present (Gilbey et al. 1982). Figure 9F, shows that remnant power in PDS of spinal animals was almost totally abolished in postganglionic sympathetic nerve and unchanged in preganglionic fibers after the ganglionic transmission had been effectively blocked by hexamethonium.

Figure 10 presents summarized values of power reduction by synaptic block within RVLM in three frequency bands: 0.4-2.0 Hz related to respiratory

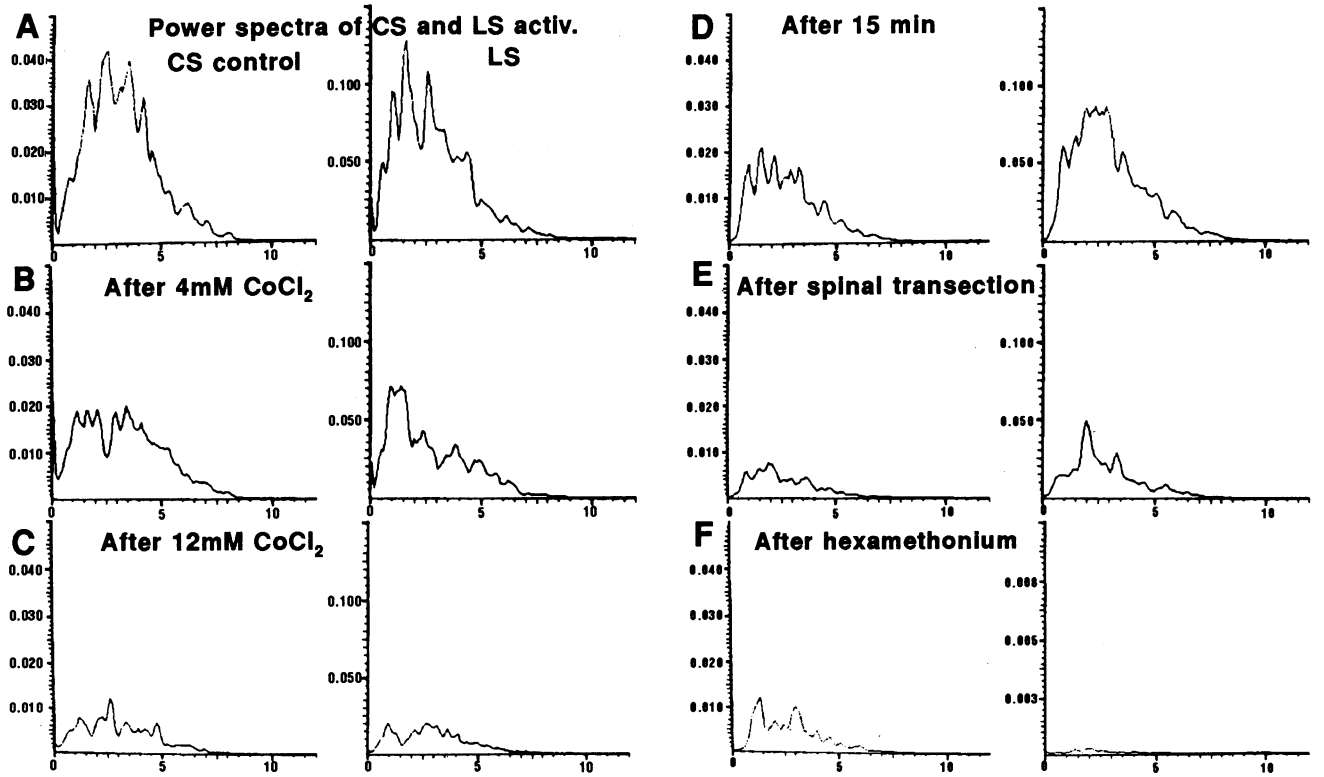

Fig. 9. A, power density spectra computed from preganglionic cervical nerve activity (CS) and from postganglionic lumbar nerve activity (LS) in the same animal before (A) and after 4 mM $CoCl_2$ microinjection into RVLM (B); after additional 12 mM $CoCl_2$ microinjection into RVLM (C); partial recovery of power in PDS after 15 min since $CoCl_2$ microinjection into RVLM (D); PDS after spinal cord transection (E); PDS after i.v. administration of hexamethonium (F).

frequency and its harmonics, 2.5-5.0 Hz intermediate frequency and in 5.5-7.5 Hz, high frequency range related to cardiac rhythm. Compared with control microinjections of saline solution, reduction of power in PDS was significant in all three frequency bands although the effect was most con-

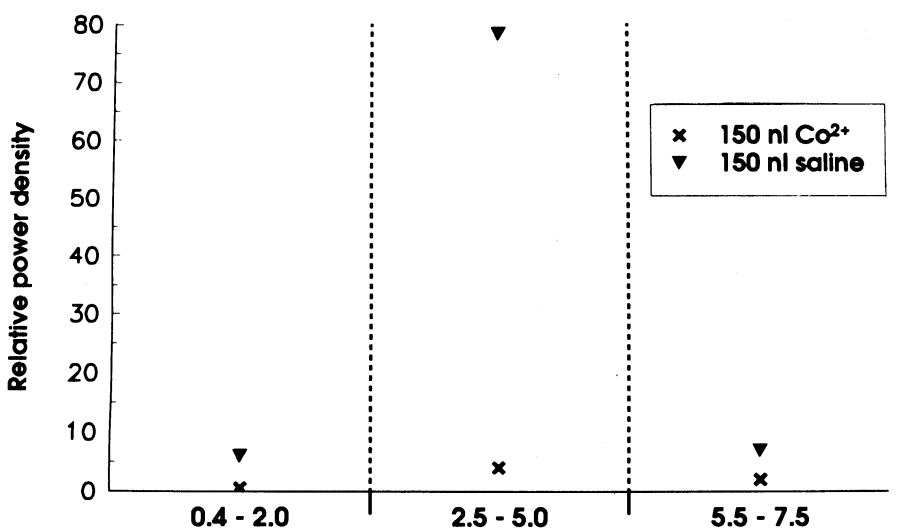

Fig. 10. Summarized mean values presenting reduction of PDS power calculated as integrated curves (surface area) in the low frequency band 0.4-2.0 Hz, intermediate frequency band 2.5-5.0 Hz and high frequency band 5.5-7.5 Hz. Control, tested after saline microinjections into RVLM, marked by triangle. Power computed after $CoCl_2$ microinjections marked by crosses. Depression of power is significant ($P<0.05$) in all three ranges of frequency.

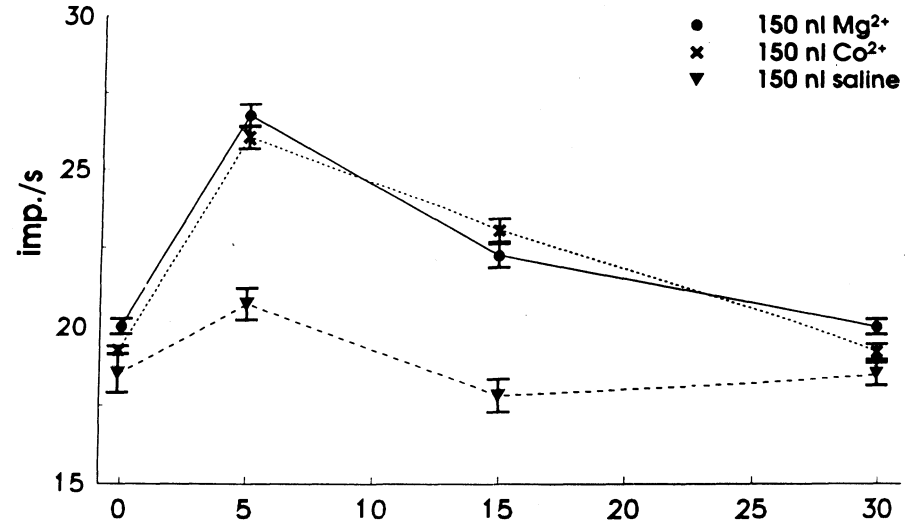

Fig. 11. Mean number of integrated spikes in all efferent sympathetic nerves under study pooled together and plotted against time in minutes. Time 0 marks microinjections of $MgCl_2$ (dots) or $CoCl_2$ (crosses) into RVLM compared to control tested by saline applied to RVLM (triangles). SE marked by vertical lines. Difference between the effects of synaptic blockers and control saline is highly significant ($P<0.001$).

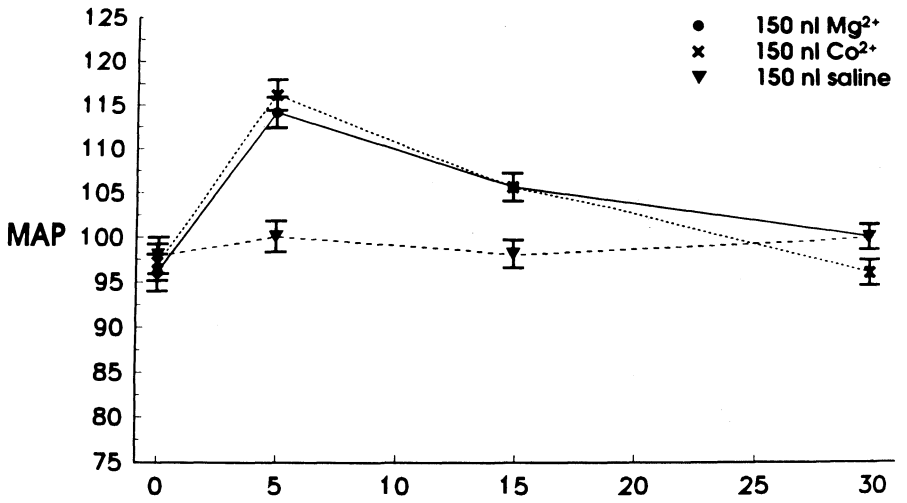

Fig. 12. After blocking of synaptic transmission to RVLM a significant pressor effect appears ($P<0.001$). Time in minutes. MAP - mean arterial blood pressure. Markings as in Fig.11.

spicuous in intermediate frequency 2.5 to 5 Hz. In control conditions most PDS power is concentrated within this frequency range.

Effect of blocking synaptic transmission in RVLM neurons on total sympathetic output, arterial blood pressure and respiratory activity

Overall sympathetic activity, measured as the number of integrated individual spikes per second increased significantly following bilateral microinjections of Co^{2+} or Mg^{2+} blockers into RVLM (Fig.11). Augmented yet desynchronized sympathetic activity corresponded to the period when time series with reduced power were computed by PDS. Parallel to the overall sympathetic excitation and desynchronization, arterial blood pressure increased and remained elevated until recovery of PDS to about initial power values (Fig.12).

No significant change in the amplitude of phrenic nerve bursts or T_I and T_E was observed during desynchronization of augmented sympathetic nerve activity and blood pressure rise (Fig.13). In contrast, microinjections of sodium kainate, a powerful neurotoxic glutamate receptors agonist,

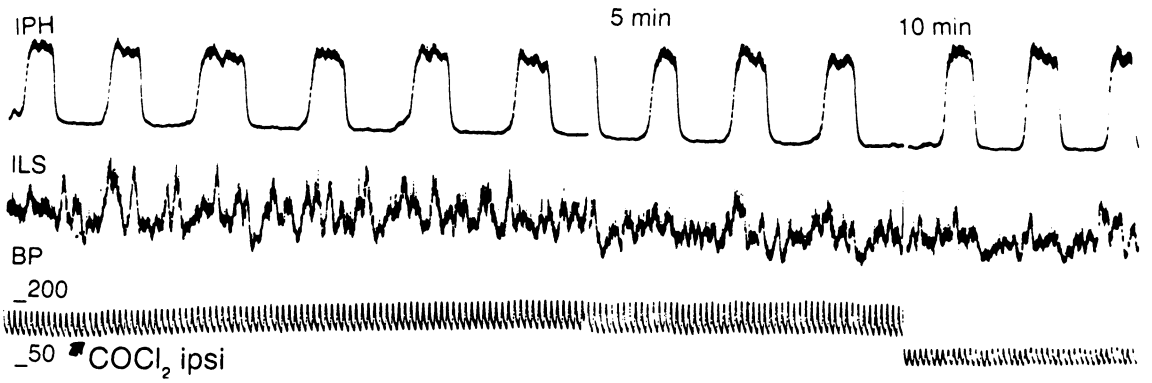

Fig. 13. Original record of the integrated phrenic nerve activity (IPH), integrated sympathetic lumbar nerve activity (ILS), and arterial blood pressure (BP) after the microinjection of 300 nl of 12 mM $CoCl_2$ into RVLM (arrow). Sympathoexcitatory and pressor effect returned to control level after 10 min. There was no significant change in the amplitude of phrenic nerve bursts and in the T_I or T_E.

produced, after transient period of strong excitation, a progressive and irreversible decrease in the sympathetic activity and blood pressure, and as well as cessation of rhythmic phrenic nerve bursts.

DISCUSSION

The efferent synchronized sympathetic nerve activity is generated centrally, as the separation of brain from peripheral reflex inputs does not abolish the sympathetic rhythm (Gebber 1980, 1990). Present results indicate that respiratory modulation of the spontaneous sympathetic discharge and its timing over respiratory cycle is coupled with respiratory brain stem oscillator via periambigual neurons. Respiratory-related propriobulbar neurons were identified in the vicinity of PAA in rats (Saether et al. 1987, Haselton and Guyenet 1989). They are likely candidates for interneurons coupling respiratory neuronal network with bulbospinal sympathoexciatatory neurons in RVLM. We suggest that six different subcategories of respiratory neurons identified in the ventral respiratory group and firing at different periods of the total respiratory cycle in rats (Schwarzacher et al. 1991) may project to the PAA interneurons. According to our hypothesis, PAA interneurons act like the gate operators filtering activity of respiratory cycle into RVLM neurons. Such a role of periambigual neurons, situated like an interphase between respiratory oscillator and sympathoexcitatory neuronal population, is supported by our findings that glutamate microinjections into discrete sites of PAA disturbed in a variable fashion the timing of maximal or minimal sympathetic activity within the respiratory cycle depending on the site at which microinjections were located in PAA (Figs.4 and 5).

Figure 14 presents a proposed neuronal model of coupling of two central oscillators, respiratory and sympathetic.

The respiratory-related rhythm of sympathetic activity accounts for only a small fraction of the total power of periodicities exhibited by spontaneous sympathetic discharges and present in PDS. A significant finding of the last years was the discovery of pacemaker activity generated by RVLM bulbospinal sympathoexcitatory neurons in the absence of major excitatory synaptic input *in vivo* (Sun et al. 1988) and in slices *in vitro* (Sun et al. 1988). Intrinsic rate of firing was high, with median rate 15 Hz, so it can not account for the synchronized efferent sympathetic nerve activity of much lower frequency, unless some spinal cord neuronal processing mechanism would filter bulbar excitatory input. Even so, any synchrony would require "cross-talk" between individual RVLM bulbospinal sympathetic neurons, a link which was not detected so far (R.M. McAllen, personal communi-

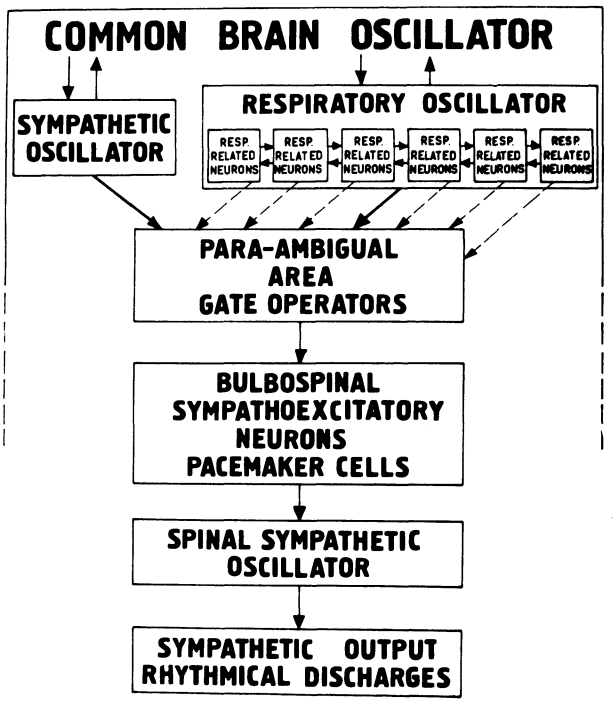

Fig 14. Schematic model of the temporal synchronization of activity between sympathoexcitatory, pacemaker neurons in RVLM and different subsets of respiratory neurons in the time domain within individual respiratory cycles. Respiratory modulation of the sympathetic discharge is mediated by the periambigual area gate-operating interneurons. Spinal sympathetic oscillator is entrained by the descending bulbospinal rhythmic respiratory-related input. Change in the synaptic transmission in the individual neurons of PAA may influence timing of the sympathetic discharge within each respiratory cycle as demonstrated by the glutamate microinjections into PAA. Koepchen's concept of common brain oscillator, encompassing coordinated sympathetic and respiratory oscillators, is visualized as a general framework. A separate subset of neuronal circuitries related more specifically to the sympathetic periodicities is presented at the upper left corner.

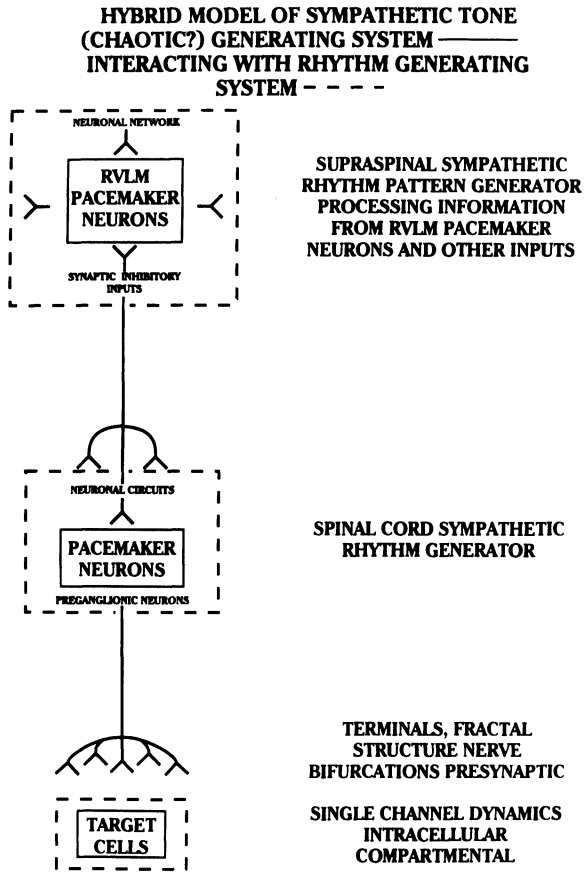

Fig. 15. Hybrid, two-component model of generation of the sympathetic activity. Chaos-like tone generating system confined to RVLM pacemaker sympathoexcitatory neurons facilitated by excitatory inputs from brain stem reticular neurons. Rhythm generating system superimposed on tone generating component *via* synaptic, mainly inhibitory, inputs into RVLM neurons. Spinal cord sympathetic oscillator entrained to rhythmicities shaped on supraspinal level. Other explanations presented in the figure.

cation). Desynchronization of efferent sympathetic activity accomplished by blocking synaptic transmission to RVLM is inconsistent with an assumption that intrinsic rhythmicity of RVLM bulbospinal sympathoexcitatory neurons provides a synchrony in sympathetic efferent discharge. Specific membrane channels responsible for pacemaker activity of RVLM bulbospinal neurons have not been identified as no "patch clamp" cell membrane studies have been so far performed on them. It appears, however, that external Ca ions are not needed for pacemaker activity of RVLM neurons (Sun et al. 1988). Therefore divalent calcium antagonists, Co^{2+} and Mg^{2+}, are unlikely to depress pacemaker activity. Divalent calcium antagonists, although unspecific, block N-type presynaptic calcium channels interrupting effectively synaptic transmission (Paton et al. 1991). A postsynaptic action of divalent calcium antagonists reduced possibly also non-NMDA glutamate receptor sensitivity. Yet, a significant and reversible sympathoexcitatory and pressor effect suggests that no

functional impairment of RVLM neurons occurred. Otherwise, a decrease in overall sympathetic activity and in arterial blood pressure would result, similar to the effects of neurotoxic glutamate receptor agonist, kainic acid microinjected into RVLM in present study. Very specific blocker of the N-type presynaptic calcium channels, a marine toxin ω-conotoxin exerts an effect which is practically irreversible thus less useful in the *in vivo* experiments. Among all advantages of divalent calcium antagonists, like short lasting effects, fast wash-out, reversibility and reproducibility, the most important one is that block in the synaptic transmission is a total one. Both excitatory and inhibitory inputs are eliminated, regardless of the kind of transmitter or membrane receptor. This feature makes divalent calcium antagonists a very efficient pharmacological tool for blocking synaptic transmission. In this regard they surpass activity of unspecific glutamate antagonist, kynurenate, as we have observed in this study (see Fig.6). Recently it has been reported that bilateral microinjections of kynurenate into RVLM in rats were ineffective in the elimination of 2- to 6 Hz sympathetic rhythm recorded in the sympathetic lumbar nerve (McAllen et al. 1993). Ineffectiveness of kynurenate and glutamate antagonists (Gebber et al. 1989) raises some doubt if glutamate receptors represent indeed the only kind of excitatory membrane receptors in RVLM neurons, a view advocated by Guyenet (1990).

Our findings are consistent with the generally accepted view that supraspinal, brain stem neuronal circuits are required for generation of synchronized sympathetic nerve rhythmicities (Gebber 1990). However, the nature of the neuronal network can not be deduced from the results presented here. According to the general idea of Koepchen (1991), multiple coordinated subsets of coupled oscillators within brain stem reticular formation are interacting in the way formalized by the theory of synergetics (Haken 1983). An inhibitory component in the central mechanism of sympathetic synchronization is evident in our experiments, since the desynchronization of sympathetic activity was accompanied by an overall increase in sympathetic discharge. One may consider the role of GABA-ergic inhibitory input from caudal ventrolateral medulla which is acting even in baroreceptor denervated animals (Zanziger et al. 1994). However, neither activation or blocking of $GABA_A$ receptors in RVLM influenced significantly synchronization of sympathetic nerve activity (McAllen et al. 1993).

Our results, which showed some though very low rhythmicity in the spinal rats, are consistent with the earlier data on synchronization of sympathetic activity in spinal cats (Mannard and Polosa 1973, Ardell et al. 1982) and with the presence of rhythmically active spinal sympathetic preganglionic neurons activated by noradrenaline in slices *in vitro* (Yoshimura et al. 1987). Contribution of spinal cord sympathetic "oscillator" to the total power of sympathetic rhythmicities computed by PDS is certainly greater in intact animals than that estimated from remnant power in PDS after acute spinalization. Facilitation and entrainment of the spinal oscillator to supraspinal excitatory input is very probable. However, an extreme view that all or most of the sympathetic rhythm of 2- to 6 Hz is generated on the spinal level and the role of the RVLM, bulbospinal sympathoexcitatory neurons is limited only to providing nonsynchronized tonic facilitatory input to the spinal cord (McAllen et al. 1993) is not compatible with our results. We were able to produce an augmented tonic sympathetic activity of minimal synchronization by interventions restricted to supraspinal level, exclusively within RVLM, a finding which is in the total disagreement with the concept of supraspinal tonic activity facilitating spinal sympathetic oscillator as the major source of sympathetic rhythm. In this regard it should be emphasized that in order to obtain a clear 2- to 6 Hz sympathetic nerve rhythm in the spinal rats McAllen et al. (1993) had to create unphysiological conditions, injecting intrathecally the neurotoxic kainic acid, normally used by us and by others for destruction and not for stimulation of neurons.

Our experimental approach enabled us to eliminate from the total sympathetic nerve activity most of the linear dynamic component computed by FFT algorithm. Augmented yet desynchronized sym-

pathetic discharges apparently are due to intrinsic pacemaker activity of RVLM bulbospinal sympathoexcitatory neurons deprived of synaptic inputs shaping out the pattern of synchronized sympathetic rhythm from nonsynchronized background activity. We postulate that this nonsynchronized activity represents a sympathetic tone upon which synchronizing neuronal circuitries superimpose their modulatory effects. In intact animals tone generating RVLM sympathoexcitatory neurons are presumably facilitated by brain stem reticular neurons which increase the total sympathetic tone (Hayes et al. 1994).

Tone-generating mechanism is evidently nonlinear. By possessing multiple nonlinear feedbacks it has apparently chaos-like attributes. It is our next step to define its non-linear characteristics. For doing so an important rule is useful: systems of self-organized criticality (Bak et al. 1987, Webber and Zbilut 1991) can be characterized numerically in frequency domain by power-law behaviour: in the frequency domain they are identified by $\frac{1}{f}$ flicker noise (intermittency, see Schuster 1984). This important law enables one to apply relatively simple log-log transformation of PDS to compute the slope of the power *versus* frequency which equals the exponent of function power - *f*. If the *f* value is less than 1, the system exhibits chaos-like behaviour. Preliminary analysis of our data suggests such kind of slope and *f*-value. Another numerical method to be applied, and also less complicated as compared to the topological approach, is the computation of Liapunow exponents in long term time series (see Schuster 1984). Both numerical methods depend, however, on the assumption that an individual time series belongs to a dynamical system devoid of parameters which drift in the long time.

In conclusion, in time domain respiratory-related periodicities of sympathetic activity are critically dependent on periambigual neuronal population, which represents an interphase included between central respiratory and sympathetic rhythm generating systems.

Our data in the frequency domain lead us to the conclusion that the central mechanism by which spontaneous sympathetic activity is organized is a two-component hybrid system. The first component, non-linear chaos-like background activity, a sympathetic tone, is generated mainly within RVLM pacemaker bulbospinal sympathoexcitatory neurons apparently facilitated by unspecific brain stem reticular activity. The second component, synchronized activity, submitted to linear FFT power spectral analysis is synaptically organized by brain stem neuronal circuits and superimposed on chaos-like tone-generating system.

Figure 15 presents two-component hybrid model, including also peripheral fractal-like complex sympathetic terminal plexuses around individual target cells and their membranes. This hypothetical scheme is only partially derived from the results of the present study and challenges rather the problems with which neurophysiologist is confronted facing the new avenue for research opened by non-linear dynamics and homeodynamics.

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