Pergamon

PII: S0306-4522(96)00445-9

Neuroscience Vol. 77, No. 1, pp. 199–217, 1997

Printed in Great Britain
0306–4522/97 $17.00+0.00

ADRENERGIC RESPONSES IN SILENT AND PUTATIVE INHIBITORY PACEMAKER-LIKE NEURONS OF THE RAT ROSTRAL VENTROLATERAL MEDULLA *IN VITRO*

A. HAYAR,* P. FELTZ and P. PIGUET

Laboratoire de Physiologie Générale, URA CNRS 1446, Université Louis Pasteur, 21 rue R. Descartes, 67084 Strasbourg Cedex, France

Abstract—Noradrenaline and adrenergic agonists were tested on pacemaker-like and silent neurons of the rat rostral ventrolateral medulla using intracellular recordings in coronal brainstem slices as well as in punches containing only the rostral ventrolateral medullary region. Noradrenaline (1–100 μM) depolarized or increased the frequency of discharge of all cells tested in a dose-dependent manner. The noradrenaline-induced depolarization was associated with an apparent increase in cell input resistance at low concentrations and a decrease or no significant change at higher concentrations. Moreover, it was voltage dependent and its amplitude decreased with membrane potential hyperpolarization. Noradrenaline caused a dose-related increase in the frequency and amplitude of spontaneous inhibitory postsynaptic potentials. The α1-adrenoceptor antagonist prazosin (0.5 μM) abolished the noradrenaline depolarizing response as well as the noradrenaline-evoked increase in synaptic activity and unmasked an underlying noradrenaline dose-dependent hyperpolarizing response associated with a decrease in cell input resistance and sensitive to the α2-adrenoceptor antagonist yohimbine (0.5 μM). The α1-adrenoceptor agonist phenylephrine (10 μM) mimicked the noradrenaline depolarizing response associated with an increase in membrane resistance as well as the noradrenaline-induced increase in synaptic activity. The α2-adrenoceptor agonists UK-14,304 (1–3 μM) and clonidine (10–30 μM) produced only a small hyperpolarizing response, whereas the β-adrenoceptor agonist isoproterenol (10–30 μM) had no effect. Baseline spontaneous postsynaptic potentials were abolished by strychnine (1 μM), bicuculline (30 μM) or both. However, only the strychnine-sensitive postsynaptic potentials had their frequency increased by noradrenaline or phenylephrine and they usually occurred with a regular pattern. Tetrodotoxin (1 μM) eliminated 80–95% of baseline spontaneous postsynaptic potentials and prevented the increase in synaptic activity evoked by noradrenaline and phenylephrine. Similar results were obtained in rostral ventrolateral medulla neurons impaled in both coronal slices and punches of the rostral ventrolateral medulla.

It is concluded that noradrenaline could play an important inhibitory role in the rostral ventrolateral medulla via at least two mechanisms: an α2-adrenoceptor-mediated hyperpolarization and an enhancement of inhibitory synaptic transmission through activation of α1-adrenoceptors located on the somatic membrane of glycinergic interneurons. Some of these interneurons exhibit a regular discharge similar to the pacemaker-like neurons and might, at least in part, constitute a central inhibitory link in the baroreceptor–vasomotor reflex pathway. Copyright © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: noradrenaline, rostral ventrolateral medulla, rat, pacemaker, interneuron, sympathoexcitatory.

The rostral ventrolateral medulla (RVL) is the medullary region with the densest population of adrenergic neurons. Moreover, it is a probable site of action of several types of antihypertensive agents which interfere with catecholaminergic transmission (for review, see Ref. 39). It was initially suggested that the origin of vasoconstrictor and cardioaccelerator sympathetic tone is due in large part to the intrinsic pacemaker activity of a small group of reticulospinal excitatory neurons located at the extreme anterior tip of the RVL (for review, see Ref. 20).

This hypothesis has been challenged by Barman and Gebber[5] who reported that sympathetic nerve discharge (SND) was not desynchronized by intracisternal injection of kynurenate in baroreceptor-denervated rats, indicating that RVL pacemaker neurons are not primarily responsible for the production of the rhythmicity in SND. Since many medullary sites were found to contain a 2–6-Hz oscillation which correlated to that in SND, it was

*To whom correspondence should be addressed at: Department of Pharmacology, School of Medicine, Box 448HSC, University of Virginia, Charlottesville, VA 22908, U.S.A.

Abbreviations: APV, (±)-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline; IPSP, inhibitory postsynaptic potential; KAc, potassium acetate; mACSF, modified artificial cerebrospinal fluid; NA, (−)-noradrenaline; PNMT, phenylethanolamine *N*-methyl transferase; PSP, spontaneous postsynaptic potential; R_{in}, cell input resistance; RVL, rostral ventrolateral medulla; SND, sympathetic nerve discharge; TTX, tetrodotoxin; UK-14,304, 5-bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine.

suggested that the oscillation in SND is an emergent brainstem network property rather than a property of the individual neurons that comprise the network.[55] It was found, however, that an excitatory supraspinal drive to the spinal cord is essential for generating the 2–6-Hz oscillation in SND, but the firing pattern of these neurons does not seem to be important for the production of this rhythmicity.[2] Therefore, the regular firing pattern of some RVL neurons is not a sufficient criterion to classify pacemaker neurons as sympathoexcitatory. A recent study by Lipski *et al.*[28a] showed that RVL presympathetic neurons recorded intracellularly *in vivo* displayed a largely irregular pattern of firing that resulted mainly from synaptic inputs, therefore supporting the "network" hypothesis for the generation of vasomotor tone.

Over 70% of the bulbospinal neuronal population of the RVL was found to be immunoreactive for the adrenaline-synthesizing enzyme, phenylethanolamine *N*-methyl transferase (PNMT). However, 38% of adrenergic neurons in this region were thought to be non-bulbospinal and might be local propriobulbar neurons.[42] The presence of barosensitive, adrenergic, non-bulbospinal RVL neurons was recently directly confirmed.[28b] Immunoelectronmicroscopic studies on the localization of PNMT support the concept that adrenergic neurons might modulate the activity of neurons containing the same or other putative neurotransmitters in the RVL. However, only a few axon terminals containing immunoreactivity for PNMT have been observed and they were mainly found to form symmetric synapses with unlabelled dendrites.[34] Moreover, there is doubt as to whether adrenaline or another catecholamine acts as a transmitter in this region since it seems that adrenaline is not stored in vesicles but is rapidly degraded by intracellular monoamine oxidases.[49,50]

The mechanism of action of catecholamines and the type of adrenoceptor as well as the neuronal phenotype involved in their cardiovascular effects are still subject to controversy. α1- and α2-adrenoceptors have been shown to mediate, respectively, the tachycardic and bradycardic responses to microinjection of adrenaline and clonidine in the intermediolateral column of the spinal cord.[32] Intracerebroventricular injection of clonidine has been reported to produce α2-adrenoceptor-mediated pressor and depressor responses in conscious and anaesthetized rats, respectively.[24] Clonidine (up to 1 μM) has been shown to produce no effect on RVL pacemaker neurons[46] and to produce an inhibitory action at a concentration of 10–30 μM through a bicuculline-sensitive mechanism.[47] Clonidine has been found to stimulate the GABAergic system in spontaneously hypertensive rats.[11] Therefore, one possible mechanism of the sympatholytic effect of clonidine could be an indirect inhibitory action through stimulation of the inhibitory brainstem networks.

We have recently recorded, in most RVL neurons examined, spontaneous postsynaptic potentials (PSPs) that often occurred in a regular pattern and were sensitive to bicuculline or strychnine.[21] As a consequence, we have hypothesized that at least some of the regular pacemaker-like RVL neurons are inhibitory interneurons and thus may not be sympathoexcitatory as it was assumed in previous *in vitro* studies.[48,46] To provide more evidence in support of this hypothesis, we investigated the effects of (−)-noradrenaline (NA) and adrenergic agonists on the membrane properties of RVL neurons as well as on the spontaneous PSPs which probably reflect the activity of the intrinsic RVL inhibitory network. Moreover, we tested for the presence of functional α1- and α2-adrenoceptors and examined whether activation of any of these receptors can affect the inhibitory synaptic neurotransmission. The present study is the first to use the isolated RVL punch preparation to test the hypothesis that the spontaneous regular PSPs originate from putative inhibitory pacemaker-like interneurons located within the RVL.

EXPERIMENTAL PROCEDURES

Wistar rats (50–100 g, Etablissement Depre) were anaesthetized with ether and decapitated. The brainstem along with the cerebellum was quickly removed and placed for 30 s in cold (2–4°C) modified artificial cerebrospinal fluid (mACSF, see below) in which sucrose 248 mM was substituted for NaCl 124 mM (see also Refs 1, 40) and equilibrated with 95% O_2/5% CO_2. The tissue was trimmed with a razor blade and a block containing the medulla and a part of the cerebellum was glued with cyanoacrylate in front of an agar block on a Petri dish and covered with the mACSF. Two to three transverse slices (400 μm thickness) containing the RVL were sectioned using an Oxford Vibratome and left to recover in mACSF at room temperature for 30 min. A slice was then transferred to the recording chamber and perfused at a rate of 1.5–2 ml/min with oxygenated ACSF (pH 7.35) of the following composition (mM): NaCl 124, KCl 2, $CaCl_2$ 2, $MgSO_4$ 2, $NaHCO_3$ 26, KH_2PO_4 1.25, and D-glucose 10. The temperature in the recording chamber was raised slowly from room temperature up to 31–32°C and the slice was allowed to equilibrate for 1 h before recording started. Signals were recorded using a high-impedance bridge amplifier (Axoclamp-2B, Axon Instruments) filtered at 10-kHz bandwidth and displayed on an oscilloscope (Tektronix 5116). The output signal was also directed to a graphic thermal recorder (Gould TA240) and a digital tape recorder (DTR-1201, Biologic) for storage and later analysis. Data analysis was performed using a personal computer equipped with a Digidata 1200 analogue/digital interface and pClamp software (Axon Inst., Foster City, CA, U.S.A.). Electrodes were filled with KCl (3 M) (30–70 MΩ) or in some experiments with potassium acetate (KAc, 2 M) (80–130 MΩ) and pH was adjusted to 7.4 with KOH and hydrogen acetate HAc, respectively.

Electrodes were guided towards the RVL with the assistance of a dissecting microscope and advanced using a micromanipulator (Narishige, Japan). The region explored is the same as defined by Li and Guyenet[28] and corresponds to that previously found to contain the highest concentration of adrenergic and other presympathetic cells (for review, see Ref. 20). Some experiments were performed on punches of slices limited to the RVL region. For that purpose, the area lying ventromedial to the compact rostral

portion of the nucleus ambiguus and lateral to the inferior olive was excised from the entire coronal slice by dissection with a razor blade under visualization through a surgical microscope. The area cut out of the medulla was a rectangle, the length of which was the floor of the medulla (approximate dimensions 1500×800 μm). This operation was accomplished as soon as the slices were sectioned and under the same conditions already described for the slicing procedure.

Drugs were dissolved in ACSF and applied via a three-way tap system, by changing the superfusion solution to one which only differed in drug content. The delay between turning the tap and the first arrival at the tissue of the exchanged solution was about 20 s. The time required to reach the concentration at equilibrium of a drug was 1 min.

The following compounds purchased from Sigma Chemical Co. (St Louis, MO, U.S.A.) were used: NA, L-phenylephrine, isoproterenol hydrochloride, clonidine hydrochloride, desipramine, prazosin, yohimbine hydrochloride, (−)-bicuculline methiodide, picrotoxin, strychnine, tetrodotoxin (TTX), and 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX). (±)-2-Amino-5-phosphonopentanoic acid (APV) and 5-bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK-14,304) were purchased from Research Biochemicals International. Sodium metabisulphite (50 μM) obtained from Research Biochemicals International was added to NA solutions to prevent oxidation.

The input resistance of RVL neurons was determined by measuring the asymptotic voltage drop caused by small hyperpolarizing currents of 100-ms duration. Changes in membrane potential due to drug application were offset by direct current injection to show the effect on membrane input resistance. However, the accuracy in evaluating the drug-induced changes in membrane conductance cannot be guaranteed quantitatively, owing to the space clamp limitations of the intracellular recording technique. Nevertheless, this should not affect any conclusion made regarding the effect of the drugs on cell input resistance (R_{in}). Spike amplitude and duration were calculated from the threshold to the peak and to the corresponding repolarization phase of the action potential, respectively. In silent neurons, these parameters were evaluated using the minimum current pulse (20 ms) needed to evoke an action potential. The resting membrane potentials were calculated upon withdrawal from cells. Statistics and curve-fitting were performed using Origin 3.0 program (Microcal Software, U.S.A.). Data are expressed as mean ± S.E.M. unless otherwise stated.

RESULTS

Basic electrophysiological properties

This work is based on stable recordings from 120 RVL neurons: 95 neurons were impaled in the whole coronal brainstem slice preparation where the RVL region can clearly be identified by its anatomical location and 25 neurons were recorded in RVL punches. The data presented here refer to two groups of neurons which we previously designated as pacemaker-like and silent, respectively.[21] Pacemaker-like neurons had a spontaneous pacemaker activity at rest with a mean frequency of 8.6 ± 0.5 Hz (range 5–14 Hz, n=35, five of them in punches), or their membrane potential was oscillating near the threshold for spike generation and injection of a small depolarizing current was sufficient to render them active with repetitive discharge and no accommodation with a minimal frequency of 7–8 Hz. Silent neurons were quiescent and did not present membrane potential oscillations at resting potential. The membrane properties of the two groups of neurons investigated in the present study have been illustrated elsewhere.[21]

The responses of these two groups of neurons to NA and adrenoceptor agonists were essentially identical at a given holding potential and no significant difference was found in either their spike duration (<1.2 ms) or their current–voltage relationship. Therefore, the data collected from these cells were considered together. The input resistance, spike duration and spike amplitude of 46 randomly chosen neurons were 114 ± 5 MΩ, 56 ± 0.8 mV and 0.91 ± 0.03 ms, respectively.

It should be mentioned here that the concentration of K^+ ions in the slice bathing solution was 3.25 mM. However, when the concentration of K^+ ions was increased to 6.1 mM, all cells displaying membrane potential oscillations (and spiking at 8 Hz with injection of a positive current, n=5) and a large proportion of the silent neurons (five out of nine tested) assumed a regular pacemaker activity. This explains why fewer impaled neurons were pacemaker than in studies by other investigators who used 6.1 mM K^+ in their superfusion solution.[48]

A third group of neurons previously termed by us irregularly firing[21] included quiescent or slowly and irregularly firing neurons with a longer action potential duration (1.2 ms). These neurons did not assume a regular pacemaker activity even with injection of positive currents. Since neurons of this group were less frequently encountered, no pharmacological investigation was performed on these cells in the present study.

Concentration and voltage dependency of noradrenaline responses

NA (1–100 μM) was applied by superfusion for 90 s to reach the equilibrium in concentration. In most cases, this time was sufficient for the membrane potential to reach a steady-state level in the presence of NA, otherwise the application was prolonged beyond this duration until the plateau of the response was obtained. Sometimes, a more pronounced depolarization was obtained upon washing NA, especially at 10 or 30 μM. As the concentration of NA was increased, the time to the peak amplitude of the response was shorter and the recovery was longer (Fig. 1A).

At a given concentration, the amplitude of the responses to NA was variable in different cells. To test whether reuptake processes could be at the origin of this variability, we examined the response to NA in control conditions and in the presence of desipramine (1 μM), which increased the amplitude and duration of the NA responses (n=7, not illustrated). In general, when tested at the resting membrane potential and in the absence of

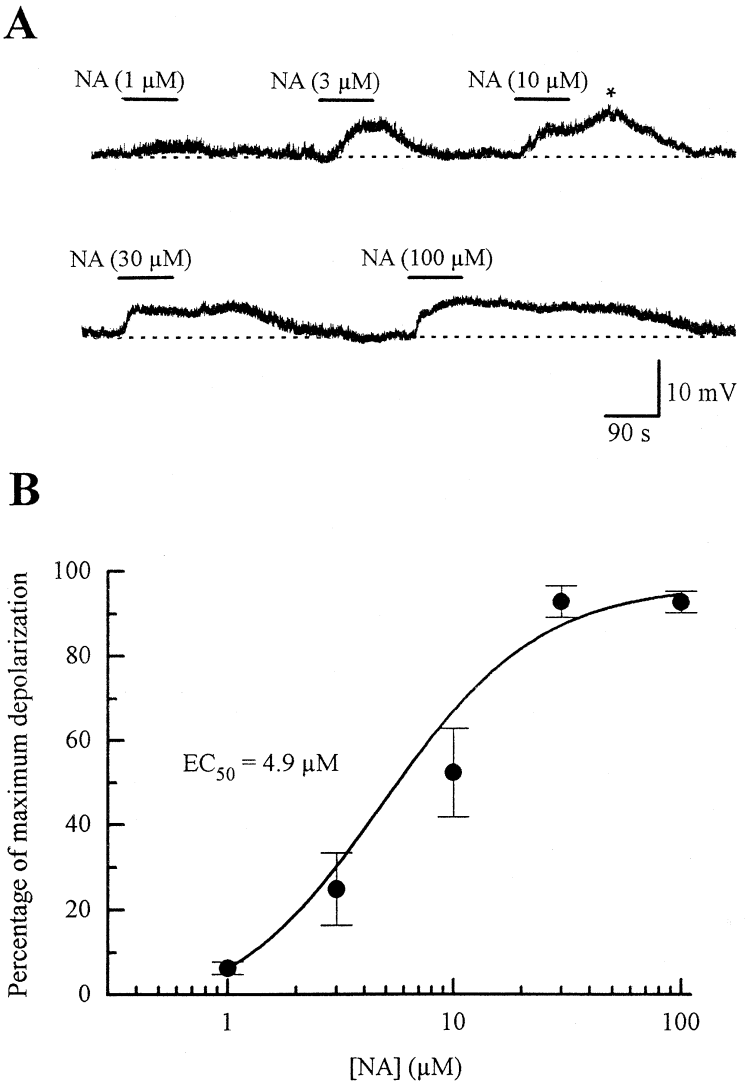

Fig. 1. Concentration-dependent response to NA. (A) Voltage recording showing a typical concentration-dependent depolarization induced by NA in a silent neuron maintained at −68 mV (dashed line) before drug application (bars). Note that a more pronounced depolarization occurs upon wash of NA at 10 μM (asterisk). (B) Concentration–response curve of the normalized depolarization induced by NA. The responses were normalized to the maximum depolarization (n =6). All cells were tested at a potential maintained 20 mV below the threshold for spike generation and the amplitude of NA depolarization was measured at the peak of the response. Error bars show the S.E.M.

desipramine, NA (10 μM) increased the firing rate of all pacemaker-like neurons tested (n=19) and this was usually associated with an increase in the slope of the depolarizing ramp during the interspike intervals. In silent neurons, NA (10 μM) evoked a depolarizing response in almost all neurons tested (n=40) at the resting membrane potential; however, in a few neurons, this response was obtained only at higher concentrations of NA.

Concentration-dependent responses to NA (1–100 μM) in the absence of desipramine were compared in six neurons whose membrane potential was maintained by continuous current injection 20 mV below the threshold for spike generation. This condition permitted us to quantify the amplitude of NA responses in the absence of neuronal spiking in silent (n=4) and pacemaker-like neurons (n=2). In Fig. 1B, the relative amplitude of the depolarizing response to NA in these six cells is plotted as a function of NA concentration yielding a half-maximal response (EC_{50}) of 4.9 μM. The maximal depolarizing response obtained in the absence of desipramine was 15 mV. At the concentrations of 3–10 μM, an apparent increase in R_{in} was observed (132 ± 22% of control, n=9). R_{in} was either not affected or slightly decreased by NA at concentrations of 30–100 μM (95 ± 15% of control, n=6).

The NA-induced depolarizing responses at either 10 μM or 30 μM were compared in four neurons at different membrane potentials from a potential just

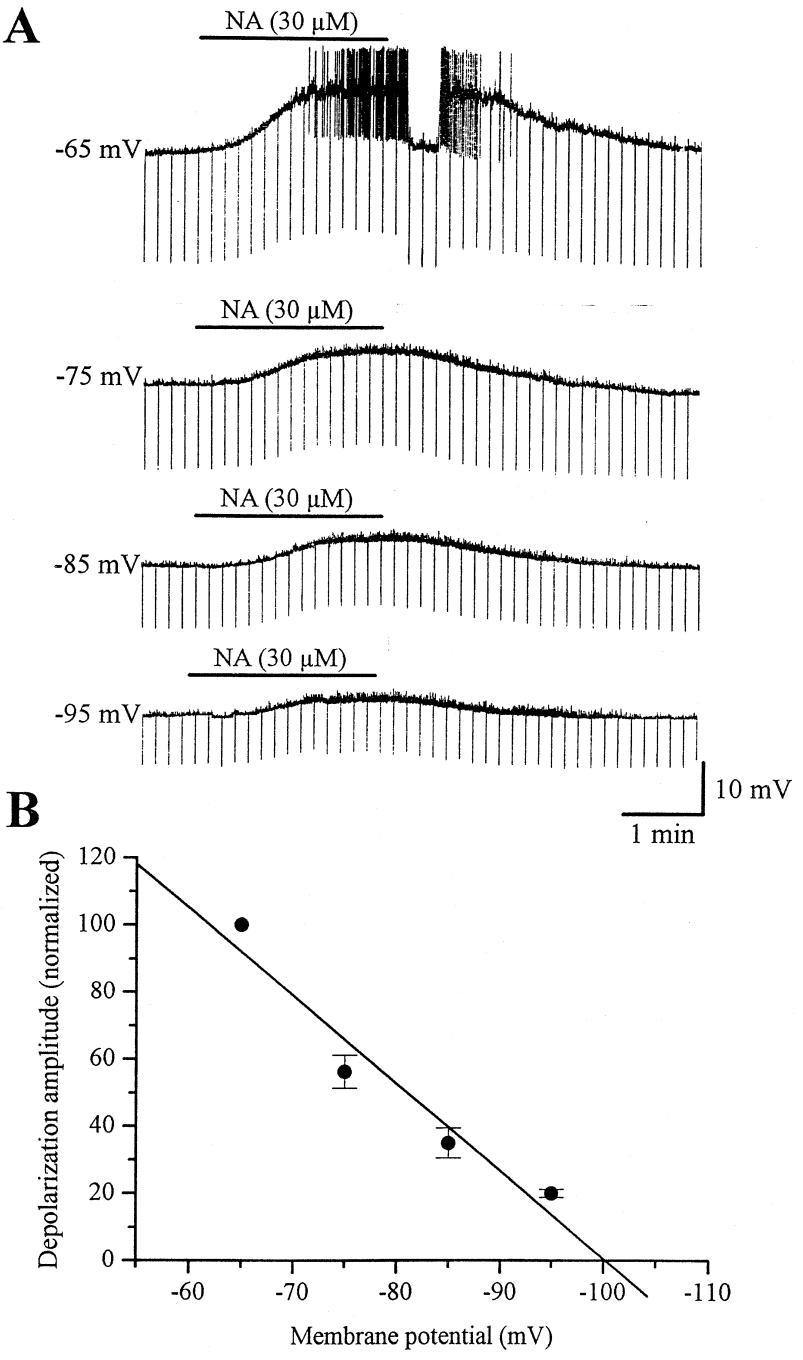

Fig. 2. Voltage-dependent response to NA in a silent neuron. (A) NA (30 μM) was applied to the neuron at different membrane potentials. Note that at −65 mV, NA depolarized the neuron and induced repetitive firing (spikes are truncated). As the membrane was hyperpolarized, the depolarization response to NA diminished. Note that this was also associated with a decrease in cell input resistance (due to an inward rectification phenomenon, see Ref. 21), as can be seen from the amplitude of downward deflections which represent voltage responses to injection of −0.2 nA current pulses (50 ms, 0.125 Hz). During the firing induced by NA in the first trace, the membrane potential was offset to control (−65 mV) by current injection for a brief period. (B) Voltage–response of the normalized depolarization induced by NA. The amplitude of the NA responses in each cell was normalized to that obtained at −65 mV. Linear regression fitting (line) indicates that NA responses are nullified at about −100 mV. Error bars show the S.E.M.

above the threshold for spike generation to more negative potentials in steps of −10 mV (Fig. 2A). When the membrane potential was shifted to more negative levels, the amplitude of the depolarizing response to NA decreased. The amplitude of this response at a holding potential of −85 mV was lower

by a factor of 3.3 ± 0.3 (n=8) than that at a holding potential of -65 mV, was further reduced at a holding potential of -90 mV (Fig. 2B), and was almost nullified at membrane potential beyond -100 mV.

Pharmacological characterization of spontaneous postsynaptic potentials

A significant proportion of RVL neurons (40%; n=48/120, silent and pacemaker-like) exhibited PSPs. There was considerable variability in the frequency and amplitude of the potentials in different neurons. These PSPs were depolarizing when recorded with KCl-filled electrodes and hyperpolarizing when recorded with KAc-filled electrodes (for illustration, see Hayar *et al.*[21]), suggesting that they were mediated by Cl^-. In some cells, especially the pacemaker-like neurons, the PSPs were small in amplitude; in these cases, a continuous negative current was injected intracellularly to enhance the magnitude of the synaptic events and increase the signal-to-noise ratio (KCl electrodes).

In silent and pacemaker-like RVL neurons recorded in both the coronal slice and the punch, we could often identify evenly spaced PSPs with a mean frequency of 8.5 ± 0.6 Hz (range 3–17 Hz, n=38, six of them in punches). A similar regularity in the firing frequency was observed in pacemaker-like RVL neurons impaled in both preparations (nearly constant interspike intervals, Fig. 3 A1, B1). The mean frequency of the PSPs in each neuron remained relatively constant over the duration of the recording (up to 4 h in some cells), indicating that presynaptic cells at the origin of the PSPs also had a fairly stable frequency. The regular pattern of spontaneous firing and PSPs is illustrated in Fig. 3 A2 and B2, respectively. The amplitude of these rhythmic PSPs was variable and ranged from just above the baseline noise level (0.4 mV) to over 25 mV when measured at a membrane potential of -90 mV. They occasionally triggered a spike at resting membrane potential. Moreover, it can be noted that there were occasionally some failures in the occurrence of PSPs, leading to a double interval between two events (Fig. 3 B1, B2).

The PSPs were not abolished by silencing pacemaker cells with negative current injection, indicating that this synaptic activity did not result from the release of neurotransmitter from the same neuron. In some neurons, two groups of rhythmic events with different mean frequencies could be identified, suggesting that the spontaneously active neurons are not necessarily synchronous (not illustrated). Nevertheless, histograms of the frequency distribution of pacemaker-like neurons and of regular events show good correlation, indicating that antecedent neurons at the origin of these synaptic events might correspond to the population of pacemaker-like RVL neurons (Fig. 3C,D). The frequency of PSPs (n=38) displayed a slightly wider distribution than the frequency of firing (n=35); however, the two distributions were centred at almost identical values (8.5–8.6 Hz, $P<0.01$).

We tested the effect of bicuculline (30 μM), strychnine (1 μM), or both, on neurons displaying spontaneous PSPs either at resting membrane potential or at a holding potential sufficiently negative to reveal measurable PSPs. Out of 21 neurons tested, bicuculline abolished all the PSPs in six neurons and some of them in three neurons; in these three cells, the remaining PSPs were abolished by additional application of strychnine. Bicuculline increased the frequency and reduced the amplitude of the PSPs in eight neurons and changed the pattern of PSPs from regular to bursting in four neurons. This bursting activity consisted of bursts of PSPs (duration 0.5–5 s) separated by silent periods. The membrane potential was reversibly depolarized by bicuculline in 16 out of the 20 neurons tested, by 6 ± 2.5 mV. This depolarizing effect was also induced by picrotoxin (100 μM, n=3).

Strychnine completely abolished the PSPs in 12 out of 20 neurons tested (Fig. 4A), and abolished some of them in three neurons. The strychnine-insensitive PSPs were abolished by additional application of bicuculline (Fig. 4B). The strychnine-sensitive PSPs exhibited in most cases a regular pattern with a mean frequency of 6.8 ± 0.9 Hz (n=10), whereas the bicuculline-sensitive PSPs were predominantly irregular. Unlike bicuculline, strychnine did not affect significantly the membrane potential of the neurons tested but progressively decreased the amplitude of the regular PSPs before they were abolished without altering their frequency or inducing a bursting pattern of PSPs.

Effects of noradrenaline and phenylephrine on spontaneous postsynaptic potentials

In almost all cells exhibiting PSPs, NA induced, in addition to a depolarizing response, an increase in baseline PSPs in a dose-related manner at concentrations from 1 to 3 μM. The major effect of NA was an increase in the frequency of inhibitory PSPs (IPSPs) which could be observed in neurons recorded with KAc-filled electrodes (Fig. 5). The regular pattern of these IPSPs was not altered during NA application. In most of the experiments, however, we used KCl-filled electrodes to reverse the polarity and increase the amplitude of IPSPs, as described above. Nevertheless, the effect of NA on baseline PSP amplitude could not be assessed because additional PSPs of different amplitudes and compound PSPs appeared during NA superfusion. The analysis of PSP amplitudes was further complicated by the high variability of PSP amplitude even in control conditions when they were regularly occurring. Moreover, since the increase in R_{in} during NA application could by itself increase the amplitude of PSPs,

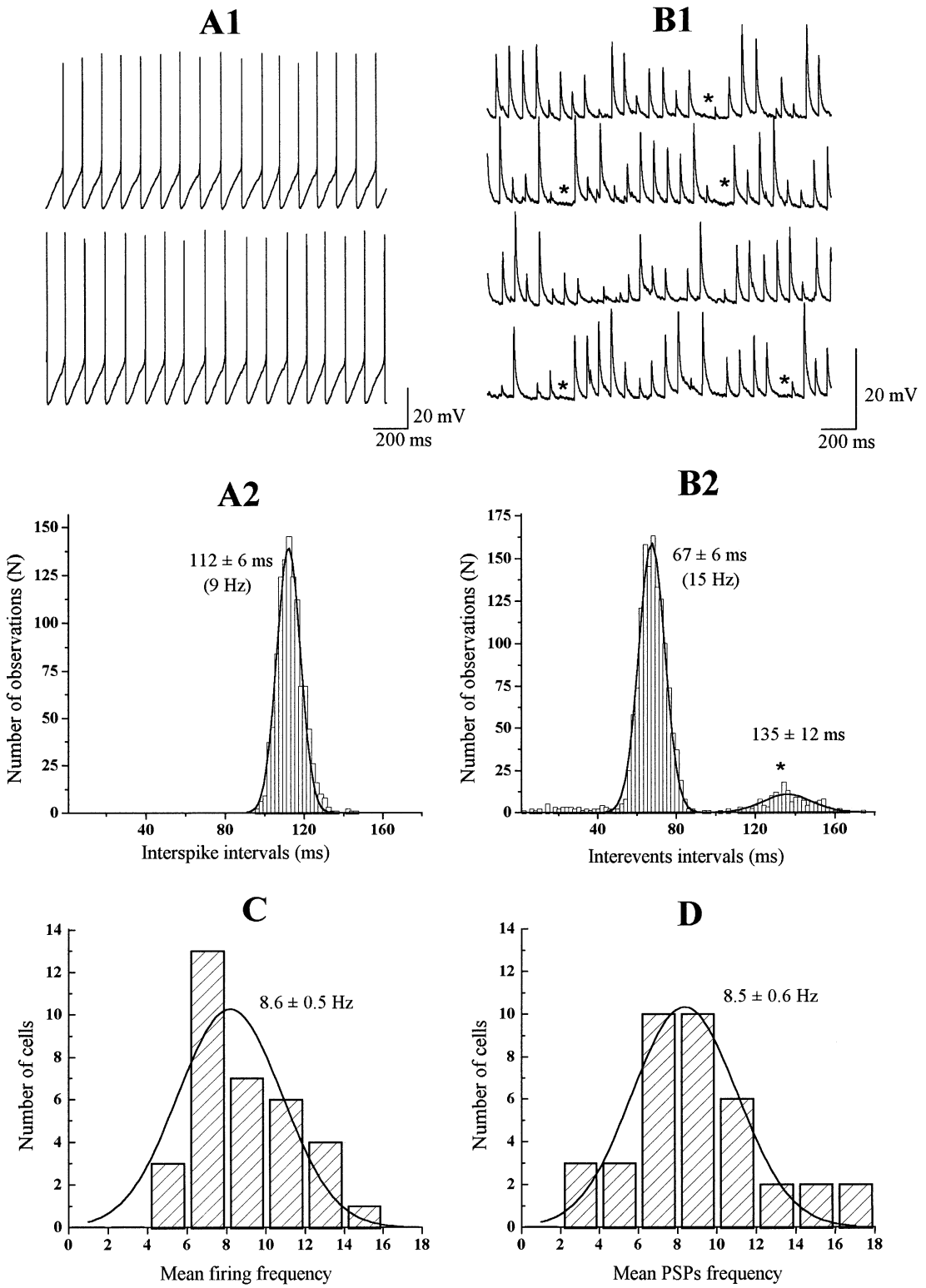

Fig. 3. Regular pattern of spontaneous discharge and spontaneous postsynaptic potentials (KCl-filled electrodes). (A1, A2) Recording from a pacemaker-like neuron and histogram of interspike intervals. The distribution of the time-intervals between action potentials was fitted by a single Gaussian distribution. The peak of this distribution occurred at 112 ± 6 ms (mean ± S.D.). (B1, B2) Regular PSPs recorded in a neuron maintained at a holding potential of -90 mV (note the variability in amplitude of the synaptic events) and histogram of interevent intervals. The distribution of the interevent intervals could be fitted with two Gaussian distributions. The midpoints of the two distributions occurred at 67 ± 6 and 135 ± 12 ms (mean ± S.D.). The second midpoint is double the first and reflects failures or small undetected events (asterisks). Data were binned at 2 ms. (C, D) Frequency distribution of the mean firing of pacemaker-like neurons and the mean frequency of PSPs. The two distributions were fitted with a single Gaussian function and centred at 8.6 ± 0.5 (n =35) and 8.5 ± 0.6 Hz (n =38), respectively (mean ± S.E.M.). Data were binned at 2 Hz.

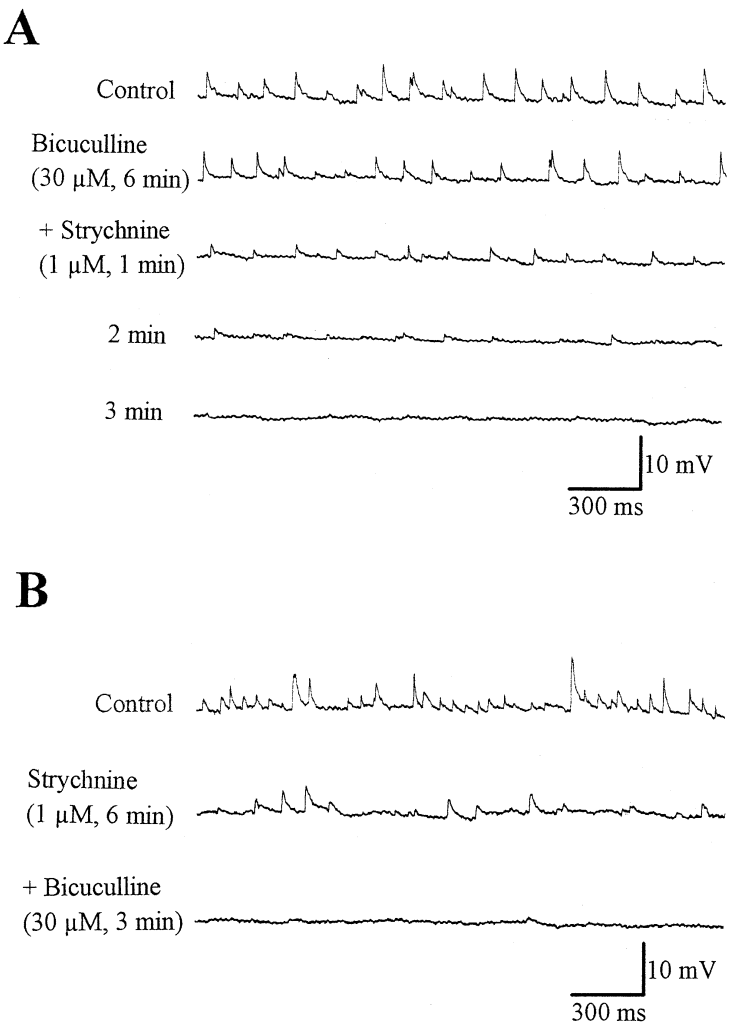

Fig. 4. Pharmacological characterization of spontaneous PSPs (KCl-filled electrodes). (A) Recordings from a neuron presenting regular PSPs that were not affected by bicuculline (30 μM) but progressively abolished by strychnine (1 μM). (B) Recordings for another neuron displaying strychnine as well as bicuculline-sensitive PSPs.

it was difficult to establish whether NA altered neurotransmitter release or affected the sensitivity of the postsynaptic receptors to the neurotransmitters. At higher concentrations (30–100 μM), NA evoked a dramatic increase in the frequency of these PSPs (Fig. 6A) and in some cases their amplitude was depressed for a brief period, probably because of an apparent decrease in R_{in}. A similar increase in baseline PSPs was observed during L-phenylephrine (10 μM) application. In 12 out of 43 neurons which did not display PSPs in control, NA (10 μM) or L-phenylephrine (10 μM) elicited the appearance of PSPs (Fig. 6B).

When bicuculline did not affect baseline PSPs, an increase in their frequency in its presence could still be induced by either NA (30 μM, n=3) or L-phenylephrine (10 μM, n=2). When strychnine abolished baseline PSPs, it prevented the increase in synaptic activity induced by either NA (n=5) or L-phenylephrine (n=4, two in punches) (Fig. 7). Application of L-phenylephrine, however, produced a slight increase in PSPs in only two out of five cells displaying bicuculline-sensitive PSPs. In all cases, NA and L-phenylephrine seemed to affect only those PSPs that occurred in a regular pattern.

NA or L-phenylephrine might increase the frequency of PSPs through an action at the level of nerve terminals by enhancing neurotransmitter release or at the level of the soma of interneurons by increasing their firing frequency. To distinguish between these two possibilities, we examined the effects of NA and L-phenylephrine in slices treated with the fast sodium channel blocker TTX (1 μM). TTX blocked 80–95% of the baseline PSPs (n=9, four of them in punches). This effect was accompanied by an inhibition of spontaneous or evoked action potentials. The residual smaller amplitude miniature PSPs occurred infrequently, indicating a low rate of action potential-independent neurotransmitter release. TTX

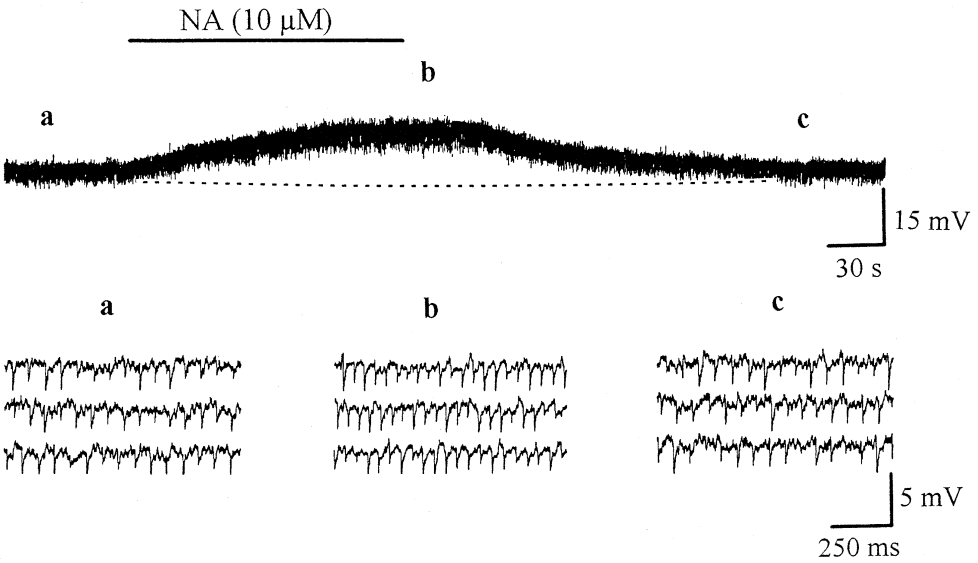

Fig. 5. Effect of NA on inhibitory PSPs (KAc-filled electrodes). NA produced, in addition to a depolarizing response (upper trace), an increase in the frequency of inhibitory PSPs without altering their regular pattern. This is shown by the expanded time-scale in the lower traces in control (a), during NA application (b), and wash (c). The resting membrane potential of this silent neuron was −64 mV.

superfusion prevented the increase in frequency of synaptic activity evoked by either NA (10 μM) (n=2) or L-phenylephrine (10 μM) (n=4, two of them in punches); however, the depolarizing response evoked by NA or L-phenylephrine persisted in the presence of TTX in all cases (Fig. 8).

To determine whether there is a polysynaptic excitatory pathway intrinsic to the RVL and involved in the generation of the PSPs, we examined the effect of excitatory amino acid receptor blockers on cells impaled in punches of RVL and exhibiting synaptic events. In four neurons tested, combined application of the *N*-methyl-D-aspartate (NMDA) receptor antagonist, APV (50 μM), and the non-NMDA receptor antagonist, CNQX (10 μM), did not affect the PSPs (Fig. 9). In three of these neurons, the PSPs occurred at regular intervals, their frequency was increased by L-phenylephrine and they were abolished by TTX (1 μM) and strychnine. The remaining neuron displayed irregular PSPs that were affected by neither L-phenylephrine nor TTX (1 μM) but abolished by bicuculline.

Effects of adrenoceptor agonists and pharmacological characterization of the noradrenaline responses

L-Phenylephrine (10 μM) was tested on 16 RVL neurons, five of which were impaled in RVL punches. All of them responded with a depolarization (6–12 mV, 8.7 ± 2.4 mV) from a holding potential maintained 20 mV below the threshold for spike generation. In all cells tested, there was an increase in the amplitude and frequency of PSPs. The response to L-phenylephrine was associated with an increase in R_{in} (142 ± 20% of control, n=8) when measured at the same holding potential as control.

In all cells tested, incubation of the slice (8–15 min) with the α1-adrenoceptor antagonist prazosin (0.5 μM) abolished the depolarizing response of NA (10–30 μM, n=16) or L-phenylephrine (10 μM, n=2) as well as the NA-evoked increase in synaptic activity (n=4) and revealed an underlying hyperpolarizing response in 10 out of 16 cells tested with NA. This latter response was small in amplitude (5–10 mV) when measured at a membrane potential that was initially maintained 20 mV below threshold for spike generation but was dependent on the dose of NA (30–300 μM) and was increased by 50 ± 15% in the presence of desipramine (0.5 μM, n=3). Additional incubation (5–10 min) with the α2-adrenoceptor antagonist yohimbine (0.5 μM) reduced this residual hyperpolarizing response by 67 ± 12% (n=5) (Fig. 10).

UK-14,304 (1–3 μM) suppressed the firing of all three pacemaker-like neurons tested and kept their membrane potential under high oscillation until enough time was allowed for recovery (10–20 min) (Fig. 11A). In contrast, it did not affect the membrane properties of four out of eight silent neurons tested at resting membrane potential; the membrane potential of the other four silent neurons was hyperpolarized by 2–5 mV and their R_{in} was slightly decreased (Fig. 11B). Clonidine (50–100 μM) suppressed the discharge and hyperpolarized the membrane potential of three out of nine pacemaker neurons, and slightly hyperpolarized three out of seven silent neurons tested. The amplitude of the hyperpolarizing response to clonidine never exceeded

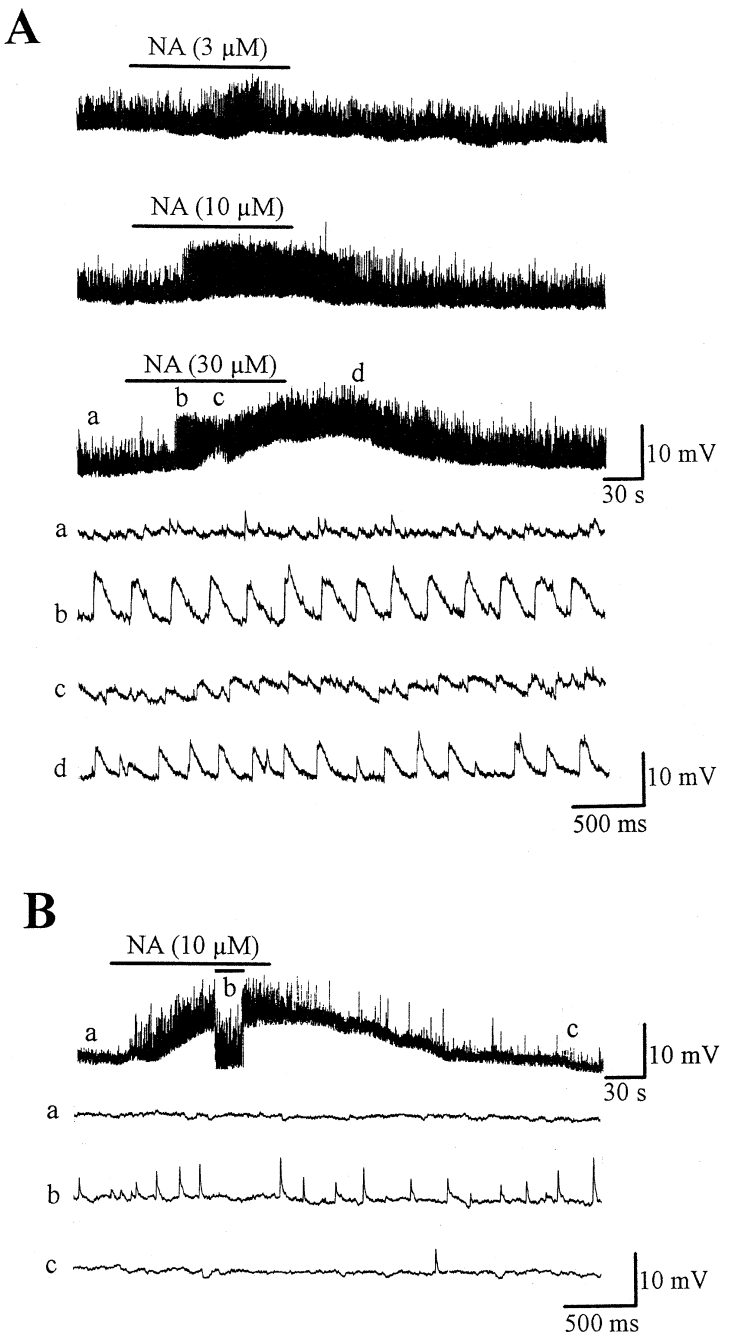

Fig. 6. NA concentration-related increase in PSPs (KCl-filled electrodes). (A) Effect of NA, 3, 10, 30 μM, on baseline PSPs in a silent neuron. Note that as the concentration increased, the increase in PSPs lasted longer. The four lower traces represent expanded time-scale of recording at the position indicated by the letters (a–d) in the trace just before. Note that additional PSPs were observed during the NA application. These PSPs were of a different time-course than those present in control (a) and their kinetic properties changed between (b), (c), and (d), probably owing to the change in R_{in}. (B) NA (10 μM) induced the appearance of PSPs (b) in another silent cell that did not exhibit any synaptic activity in control (a). These PSPs disappeared upon washing to control solution (c). In (b), the membrane potential was offset to control by current injection.

4 mV. Clonidine slightly decreased the frequency of PSPs by 20–30% in four neurons tested. Isoproterenol (10–30 μM) affected neither the resting membrane potential of six silent neurons tested (Fig. 11C) nor the spiking frequency of two pacemaker-like neurons. Only in one neuron was an increase in PSPs observed after isoproterenol application.

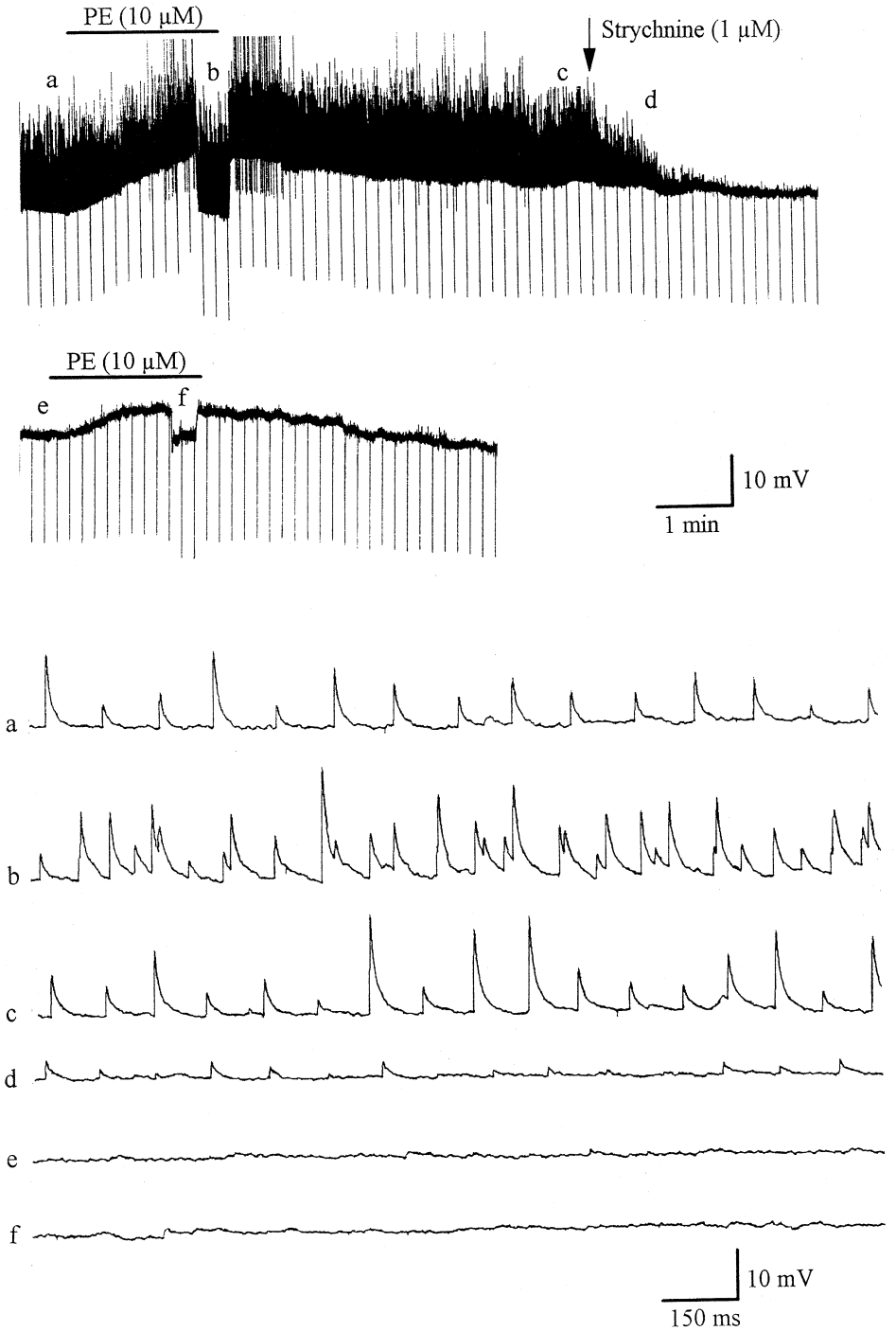

Fig. 7. Pharmacological characterization of PSPs evoked by superfusion of L-phenylephrine (KCl-filled electrode). Effect of L-phenylephrine (10 μM) on a RVL neuron presenting regular PSPs and held at a potential of −80 mV with constant negative current injection. L-Phenylephrine was tested in control and in the presence of strychnine (1 μM). The five lower traces represent expanded time-scale recordings of control (a), during L-phenylephrine application (b), wash (c), during perfusion with strychnine (d) and (e), and L-phenylephrine plus strychnine (f). Note that L-phenylephrine increased the frequency of PSPs and induced the appearance of compounds PSPs, probably owing to the recruitment of additional nerve terminals. These PSPs were progressively inhibited by strychnine. The depolarization induced by L-phenylephrine persisted in the presence of strychnine, which abolished the PSPs even during L-phenylephrine application. Note the increased R_{in} which was monitored by injecting negative current pulses (duration 50 ms, amplitude −0.2 nA, 0.125 Hz). The high-amplitude events are truncated spikes induced by the PSPs. PE, L-phenylephrine.

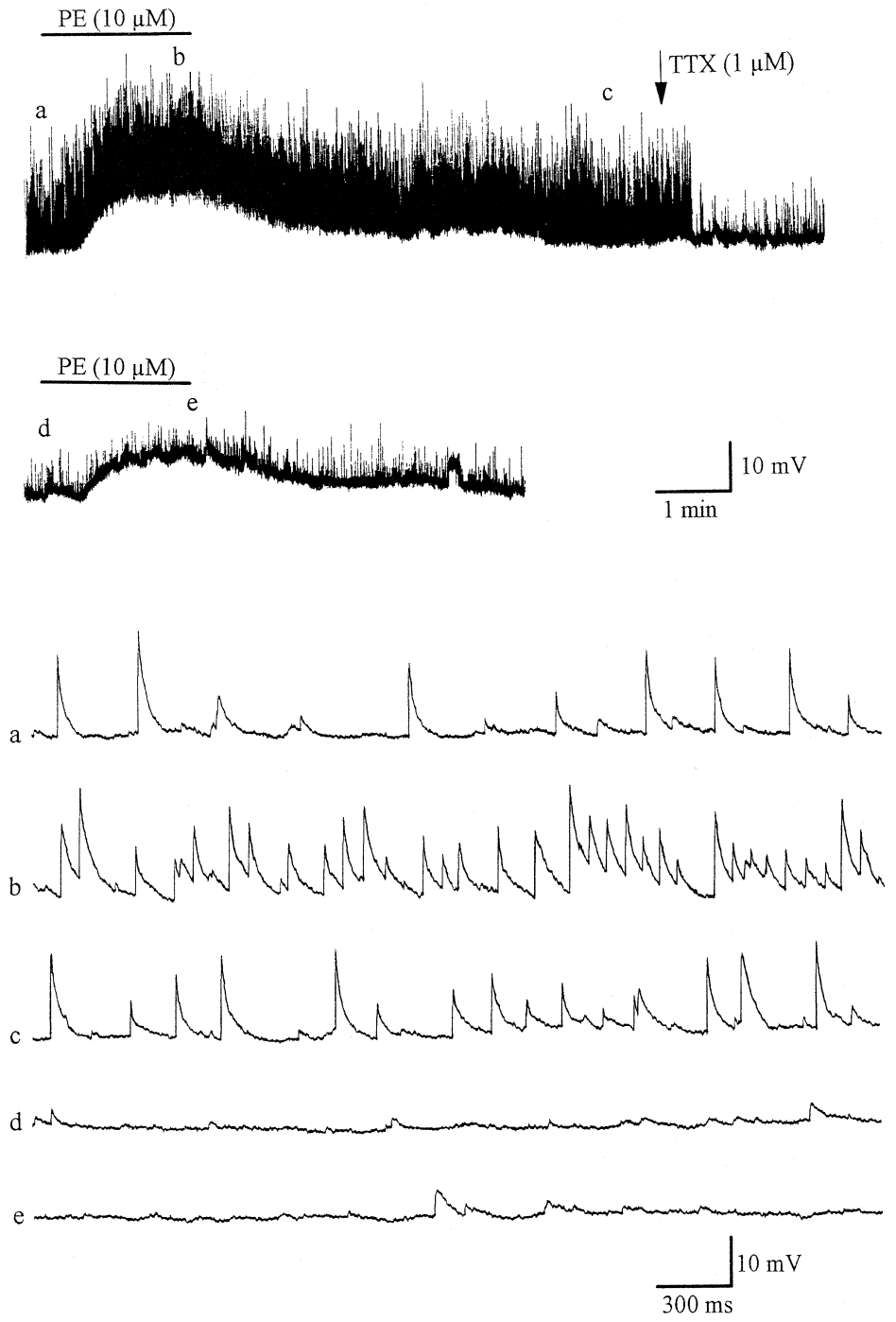

Fig. 8. Sensitivity of L-phenylephrine-evoked PSPs to TTX (KCl-filled electrode). Effect of L-phenylephrine (10 μM) on a RVL neuron presenting relatively regular PSPs and held at a potential of −85 mV with constant negative current injection. L-Phenylephrine was tested in control and in the presence of TTX (1 μM). The lower five traces represent selected recordings at an expanded time-scale of control (a), during L-phenylephrine application (b), wash (c), in TTX (d) and L-phenylephrine plus TTX (e). Note that after perfusion with TTX, which eliminated the majority of PSPs, L-phenylephrine no longer elicited an increase in the frequency of miniature PSPs; however, the depolarization induced by L-phenylephrine persisted in the presence of TTX. PE, L-phenylephrine.

DISCUSSION

In this study, we have presented evidence that supports the existence of functional α1- and α2-adrenoceptors on silent and pacemaker neurons of the RVL. Our data show, moreover, that selective activation of α1-adrenoceptors facilitates inhibitory transmission via stimulating inhibitory interneurons. Some of these interneurons may have a regular

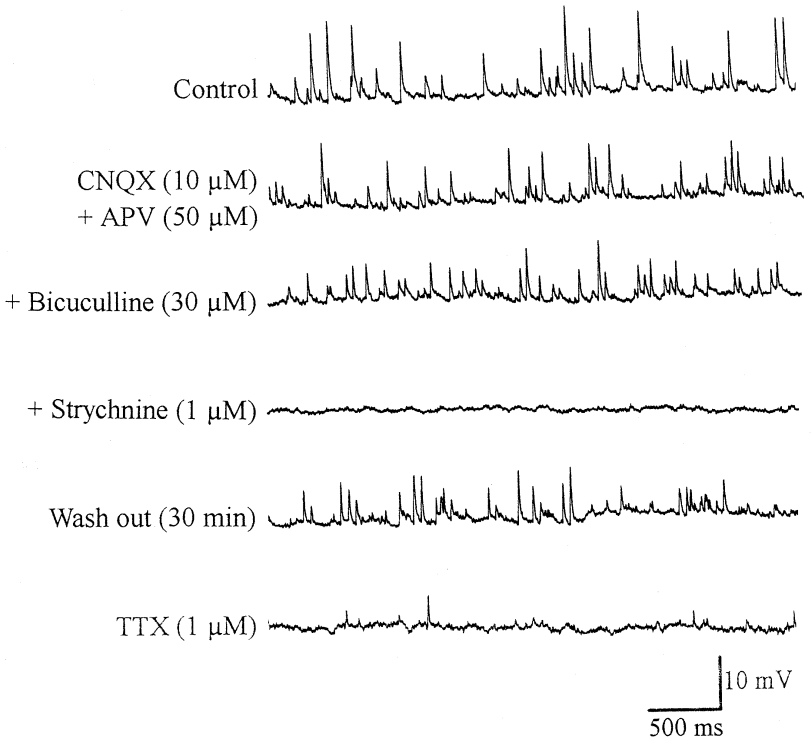

Fig. 9. Lack of effect of excitatory amino acids on PSPs recorded in a neuron impaled in RVL punch (KCl-filled electrode). The PSPs were not affected by combined application of CNQX and APV. They were insensitive to bicuculline but completely and reversibly abolished by strychnine. TTX eliminated the majority of these PSPs. All recordings were obtained from the same neuron.

discharge activity similar to pacemaker-like neurons, suggesting that the latter could be responsible for the regular pattern of IPSPs. Finally, using RVL punches, we demonstrated that these regularly discharging interneurons have their soma located within the RVL.

α_1- and α_2-adrenoceptor-mediated effects

There were no apparent differences between silent and pacemaker-like neurons regarding their responsiveness to α1- and α2-adrenoceptor agonists. The two adrenoceptor subtypes were found to mediate opposing effects, and may co-exist on the same neuron. This phenomenon is not unique to the RVL since multiple effects of NA both on different neurons and on the same neuron have been reported for sympathetic preganglionic neurons as well as many other neurons in the brain, e.g., hippocampal pyramidal neurons and granule cells of the dendate gyrus (for reviews, see Refs 9, 53).

No evidence was obtained for the existence of functional β-adrenoceptors in the RVL. We are unable to explain the discrepancy between our results and those of Sun and Guyenet[46] who found that *in vitro*, RVL pacemaker neurons were activated by isoproterenol (10 μM) and unaffected by L-phenylephrine (up to 100 μM). However, only a few intracellular recordings were performed in their study; therefore, there may be some doubt as to the type of neurons they investigated. Moreover, in the same laboratory, Allen and Guyenet[3] later found that iontophoretic application of isoproterenol in the RVL *in vivo* produced no effect on any of the cells studied.

In our study, the depolarization evoked by NA is probably due to activation of α1-adrenoceptors since it was blocked in all cases by the α1-adrenoceptor antagonist prazosin and was mimicked by the α1-adrenoceptor agonist L-phenylephrine and not by the β-adrenoceptor agonist isoproterenol. The associated increase in R_{in}, which could be due to a decrease in potassium conductance, further supports the involvement of α1-adrenoceptors in mediating the response, as has been reported similarly for sympathetic preganglionic neurons[53] and dorsal raphe neurons.[37] This is supported by the fact that we determined an extrapolated reversal potential of the NA depolarizing response at −100 mV which is close to the equilibrium potential of K^+ ions. The NA- and L-phenylephrine-induced depolarizations seem to involve a postsynaptic mechanism since they persisted in the presence of TTX. The observation that TTX prevented the increase in PSPs in response to NA and L-phenylephrine also supports the hypothesis that α1-adrenoceptors are localized on the soma of interneurons rather than on the nerve terminals.

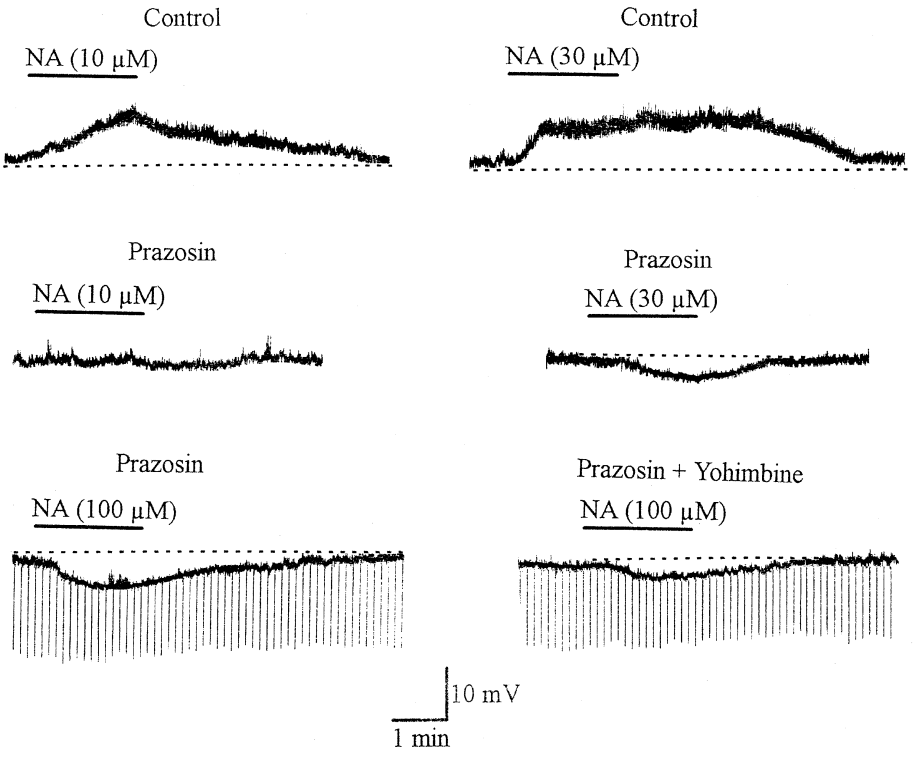

Fig. 10. Pharmacological characterization of the NA response (KCl-filled electrode). Effects are shown of NA (10 and 30 μM) in control, NA (10, 30 and 100 μM) in prazosin (0.5 μM) and NA (100 μM) in the presence of prazosin (0.5 μM), and yohimbine (0.5 μM). All recordings in this figure were from the same cell. NA produced a dose-dependent depolarization and hyperpolarization in control and prazosin, respectively, and yohimbine partially reduced the NA hyperpolarization. The NA hyperpolarization was associated with a small decrease in R_{in} monitored by injecting negative current pulses (duration 50 ms, amplitude −0.2 nA, 0.125 Hz). Note that prazosin prevented the NA-induced increase in PSPs observed in control conditions.

To our knowledge, there has been no clear demonstration so far of a specific role of α1-adrenoceptors in the rat RVL itself. When injected in the RVL, NA and adrenaline have been found to lower arterial pressure and heart rate, while the α1-adrenoceptor agonist L-phenylephrine, which is also a weak α2-adrenoceptor agonist, produced a small but significant pressor effect at low doses and elicited a slight fall in arterial pressure at higher doses.[13] The potent α1-adrenoceptor agonists cirazoline and ST 587 produced dose-dependent hypotensive effects when injected in the nucleus reticularis lateralis of the cat.[8] It is possible that the effect of these substances resulted from the activation of distinct receptor types with different affinity when using the microinjection technique. Nevertheless, the attribution of a sympathoinhibitory role for the α1-adrenoceptors in the ventrolateral medulla is supported by a recent finding that NA injection in the ventrolateral depressor area (containing mainly GABAergic neurons projecting to the RVL) produced a depressor and bradycardic response that was blocked by prazosin.[31]

In the presence of prazosin, a large proportion of RVL neurons was hyperpolarized by NA (30–300 μM) and this effect was associated with a decrease in R_{in}. The latter observation could explain why no significant change in R_{in} was obtained with high concentrations of NA in control conditions and suggests that an α2-adrenoceptor-mediated decrease in R_{in} at relatively high concentrations of NA could counteract the increase in R_{in} induced by α1-adrenoceptor activation. The existence of an α2-adrenoceptor-mediated response would suggest that some of these neurons might belong to the C1 adrenergic group, since immunoreactivity for α_{2A}-adrenergic receptors has been detected in almost all the PNMT-immunoreactive cells of the RVL.[41] Consistent with this suggestion is a recent study demonstrating PNMT immunoreactivity in a significant proportion of both silent and pacemaker RVL neurons.[22] This would indicate that pacemaker or adrenergic neurons are not necessarily a functionally homogeneous population.

The NA hyperpolarizing effect was only partially reduced by yohimbine and was small in amplitude. It is possible that this resulted from a high level of uptake of NA since the amplitude of the hyperpolarization was increased in the presence of desipramine. Moreover, the α2-adrenoceptor agonists UK-14,304 and clonidine had modest but significant effects on the membrane properties of RVL neurons. These substances generally suppressed

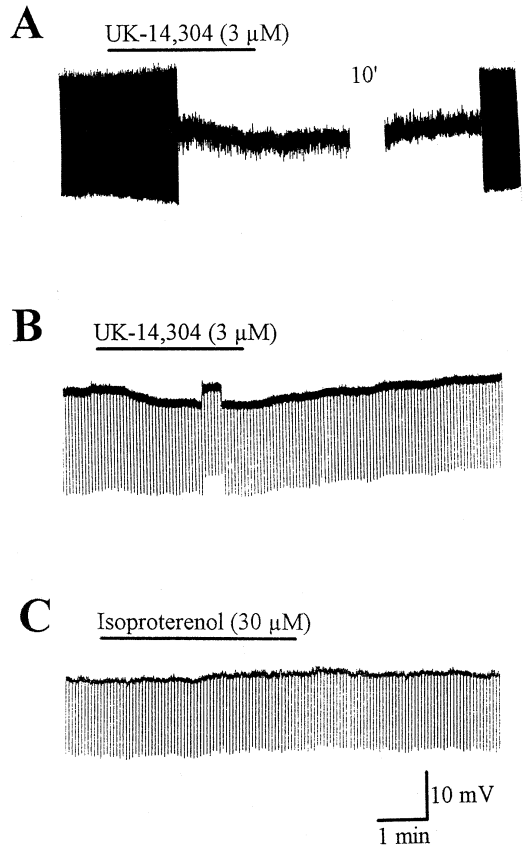

Fig. 11. Effect of UK-14,304 and isoproterenol on membrane properties of pacemaker and silent neurons. (A) UK-14304 (3 μM) silenced a pacemaker neuron and the membrane potential remained under high oscillation until enough time was allowed for recovery (15 min). (B) UK-14,304 (3 μM) hyperpolarized a silent neuron and decreased its R_{in}. During the application the membrane potential was restored to control (−65 mV) to show the effect of R_{in}. (C) The membrane properties of another silent neuron (resting potential −63 mV) were not affected by isoproterenol (30 μM). Downward deflections in B and C are voltage responses to injection of negative current pulses (duration 50 ms, amplitude −0.2 nA, 0.25 Hz).

the discharge of pacemaker neurons while only slightly hyperpolarizing the membrane potential of some pacemaker and silent neurons. Only high concentrations of clonidine (10–30 μM) were found to be able to silence RVL pacemaker neurons in a similar *in vitro* study.[47] It is possible that, in addition to activating α2-adrenoceptors, there is a non-selective activation of dopamine D_2 receptors by NA, as has been reported in dopamine midbrain neurons.[17] However, this possibility was not addressed in this study.

Pharmacological characterization of the receptors mediating the noradrenaline-induced enhancement of synaptic activity

Monoamine-induced increases in IPSPs have been reported in many areas of the CNS. NA increased GABA-mediated IPSPs in the substantia gelatinosa of guinea-pig spinal trigeminal nucleus,[18] in the pyramidal cells in piriform cortex,[15] and in CA1 pyramidal neurons of the hippocampus.[30] L-Phenylephrine increased GABA-mediated IPSPs in midbrain dopamine neurons.[17] Adrenaline elicited glycine-mediated IPSPs in sympathetic preganglionic neurons.[35]

Our results suggest that at least some of the neurons depolarized by NA or L-phenylephrine via α1-adrenoceptors are glycinergic interneurons, probably located within the RVL, the excitation of which results in the increase in strychnine-sensitive PSPs. Furthermore, TTX blocked the NA and L-phenylephrine-induced increase in PSPs exhibited by RVL neurons in the coronal slice as well as in RVL punches, suggesting that the excited neurons are located within the RVL. The additional contribution of glycine-projecting neurons to the RVL from adjacent nuclei in the slice, e.g., nucleus tractus solitarius or raphe nuclei, cannot be excluded since the RVL was shown to have bilateral connections with many RVL nuclei.[54] However, an anatomical substrate supporting the presence of glycine RVL interneurons or glycinergic neurons projecting to the RVL is still lacking. Nevertheless, moderate levels of binding of [^{3}H]strychnine have been demonstrated in the RVL and paragigantocellular nucleus[52] and the glycine transmitter transporter 2 mRNA is moderately expressed in the gigantocellular reticular nucleus.[29]

The regular pacemaker neurons recorded in this study were probably responsible for the action of NA or L-phenylephrine on the regular glycine-mediated PSPs. This is supported by our finding that NA or L-phenylephrine depolarized the regular pacemaker neurons and increased their firing rate. Moreover, PSPs occurring at regular intervals and characterizing pacemaker activity were in most cases inhibited by strychnine at a concentration of 1 μM, whereas bicuculline (30 μM) increased their frequency and reduced their amplitude only at higher concentrations (100–150 μM). In contrast, bicuculline-sensitive PSPs were either slightly increased or not affected by NA or L-phenylephrine and they usually had an irregular pattern. One possible explanation is that most of them are miniature events caused by spontaneous release of GABA from nerve terminals and that only action potential-dependent events are affected by the α1-adrenoceptor agonists.

The exact mechanism involved in the bicuculline-induced depolarization remains to be established. This depolarization could not be interpreted as a non-specific effect of bicuculline since it was mimicked by picrotoxin but not by strychnine. Therefore, this observation supports the presence of a tonic GABAergic inhibition within the RVL. The present study provides evidence for a higher contribution of glycine than GABA receptors in mediating the

inhibitory transmission, at least in the neurons investigated. Diffusely scattered terminals and numerous perikarya containing the synthetic enzyme for GABA, glutamate decarboxylase, have been identified immunocytochemically within the RVL.[33,43] In the ventral horn of the rat spinal cord, all glutamate decarboxylase-positive terminals were found to be in direct apposition with glycine receptors.[51] GABA, glycine and hybrid GABA–glycine receptors, however, appeared to mediate the responses to GABA and glycine applications in embryonic cultured rat medullary neurons, with all three receptors sensitive to blockade by both glycinergic and GABAergic antagonists.[25] It is tempting to suggest that GABA, either alone or in combination with glycine, may be endogenously released to activate strychnine-sensitive glycinergic receptors. It is also possible that GABAergic neurons innervating the RVL are predominently located in the caudal ventrolateral medulla (a region not included in our slice preparation), while the majority of RVL inhibitory interneurons are glycinergic. Alternatively, $GABA_A$ receptors synapses may be located electrically distal to the intrasomatic electrode, a location that could make bicuculline-sensitive PSPs undetectable.

The regular pattern of inhibitory glycinergic PSPs does not seem to result from a polysynaptic pathway involving pacemaker excitatory neurons. First, when using KAc-filled electrodes, depolarizing PSPs were almost never observed at resting membrane potential, suggesting that there were few excitatory PSPs. Second, combined application of the excitatory amino acid receptor blockers, CNQX and APV, did not affect the regular TTX-sensitive PSPs in the RVL punches. This indicates the predominant inhibitory nature of the synaptic events and also suggests that they are unlikely to be due to a polysynaptic mechanism involving excitatory spontaneously active interneurons. This is supported by the observation that injection in the RVL of non-selective tracers labelled numerous neurons around the injection sites, whereas [^{3}H]D-aspartate injections labelled only a few neurons in the RVL,[44] suggesting that the majority of interneurons in the RVL are inhibitory.

Functional considerations

Taken together, our data suggest that more than one mechanism may operate to mediate the sympatholytic action of catecholamines when injected in the RVL. This could be accomplished by a direct inhibition via α2-adrenoceptor-mediated hyperpolarization or by facilitating inhibitory synaptic transmission via α1-adrenoceptors located on the soma of inhibitory interneurons. We cannot ascertain that these direct and indirect inhibitory actions produce sympatholytic actions in physiological conditions since the sympathoexcitatory nature of the responding neurons cannot be demonstrated in our *in vitro* experiments.

Several lines of evidence suggest that the source of catecholamines released in the RVL is endogenous. Blessing and Willoughby[7] proposed that neurons in the area postrema are the only catecholamine-synthesizing cells in the medulla and pons with projections to the RVL (C1-area) in the rabbit. This means that neither the A5 neurons[45] nor the A1 neurons of the caudal ventrolateral medulla[16] project to the RVL (C1 region), as has been suggested by previous studies. Cunningham *et al.*[10], however, found that the axons of the area postrema that traverse the region of the C1-cells group make few arborizations, suggesting little functional contact. Therefore, it is most likely that the adrenergic receptors in the RVL are apposed to nerve terminals or dendrites of local adrenergic neurons.

Furthermore, our results support the presence of glycinergic regularly firing interneurons within the RVL in the *in vitro* slice preparation. This hypothesis is supported by the presence of strychnine- and TTX-sensitive PSPs with regular frequency in the majority of RVL neurons recorded in the coronal slice as well as in punches containing only the RVL region. It is also interesting to notice that the mean firing rate of the pacemaker neurons and the mean frequency of the PSPs were not significantly different and both were increased by L-phenylephrine and NA by activating α1-adrenoceptors.

The RVL is the original ''glycine-sensitive area'' described by Guertzenstein and Silver.[19] Glycine decreases the blood pressure when microinjected in the RVL of the rat; however, strychnine has no effect.[4] Glycine has been shown to mediate baroreceptor inhibition of sympathetic activity at a spinal site.[26] However, GABAergic but not glycinergic receptors were shown to mediate the depressor response evoked by activation of the caudal ventrolateral medulla.[6] Nevertheless, detection of glycine release in the ventrolateral medulla by the microdialysis technique *in vivo* provides evidence for a physiologically relevant role of glycine in this region of the brain.[23] The involvement of glycine in cardiovascular regulation has recently been proposed by Moriguchi *et al.*,[36] who found that the attenuation of the baroreflex by angiotensin II was associated with an augmented release of this amino acid in the RVL. Moreover, an increase of glycine release from the ventral medullary surface occurred after baroreceptor stimulation.[12] Glycine-mediated inhibition was also found to be vital for developmental maturation of the respiratory network within the ventrolateral medulla.[38] However, glycine might not be as important as GABA in mediating the tonic sympathoinhibition within the RVL as shown by previous *in vivo* studies.[4] Therefore, it is possible that the majority of the neurons investigated in our study, which receive a tonic glycinergic input, might be propriobulbar neurons. This is supported by

the finding that the reticulospinal projection is represented by a remarkably small number of RVL neurons, around 200 in the rat.[42]

The silent and pacemaker-like RVL neurons could not be distinguished according to the membrane electrophysiological and pharmacological properties examined in this study. Therefore, it is possible that these neurons belong to a functionally homogeneous population but have different levels of membrane excitability ranging from completely silent, to oscillating, to spontaneously active. Since some neurons were capable of generating a rhythmic pattern of discharge when appropriately depolarized, when the bathing extracellular K^+ ion concentration was increased, or in the presence of NA, it is difficult to determine whether spontaneously active neurons are true or "conditional pacemakers" (for discussion, see Ref. 20).

We have previously characterized in the RVL a group of irregularly firing neurons with distinct electrophysiological properties, namely a relatively low discharge rate, longer spike duration and the presence of an inward rectifying current.[21] Recently, only RVL neurons with relatively slow and irregular firing were found to be inhibited significantly by clonidine *in vivo*[2] and by the α2-agonist UK-14,304 *in vitro*.[28] Our unpublished data also indicate that the majority of irregular firing neurons were hyperpolarized by NA, suggesting that they possess predominantly α2-adrenoceptors.

Although the neuronal circuit mediating the responses to catecholaminergic agonists might be very complex, we propose a simple hypothetical model for this circuit (Fig. 12). We suggest the existence of at least three types of RVL neuron: silent (50%), pacemaker-like (25%), and irregular firing (25%), based on our previous study.[21] Silent and pacemaker-like neurons could be mainly inhibitory interneurons containing essentially glycine, whereas irregular firing neurons could be the putative C1 adrenergic neurons. However, glutamate might be the fast neurotransmitter co-localized with catecholamines and released in the spinal cord by the adrenergic neurons. Although the excitatory spinal projection constitutes the greatest proportion of bulbospinal RVL neurons, it is possible that some local inhibitory neurons also project spinally to inhibit sympathetic preganglionic neurons. α1- and α2-adrenoceptors are predominantly located on pacemaker-like and irregular firing neurons, respectively. The excitability of C1 adrenergic neurons could be limited by inhibitory somatic α2-adrenergic autoreceptors. NA might be also released by these neurons to stimulate α1-adrenoceptors located on glycinergic interneurons which, in turn, inhibit all types of RVL neuron. Finally, we would like to mention that this model is still highly speculative and should be considered carefully until direct evidence for receptor distribution and cell connectivity is provided in future studies.

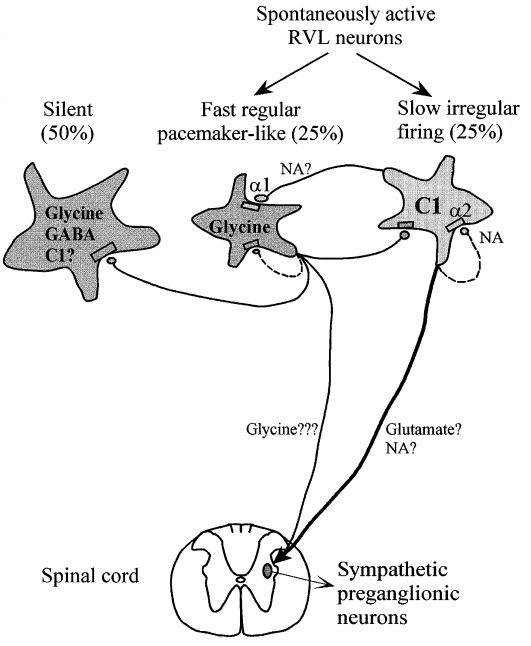

Fig. 12. A simple hypothetical model indicating the types of RVL neurons, the presumed location of the α1- and α2-adrenoceptors, the cell connectivity, and the putative sympathoexcitatory and sympathoinhibitory pathways. Each group of RVL neurons is represented by one single neuron, therefore recurrent connections (dashed line) imply mutual connections between neurons of the same group and not autapses. In this model, NA activates inhibitory α2-adrenoceptors located on the soma of C1 adrenergic sympathoexcitatory neurons, and inhibits these neurons indirectly by enhancing inhibitory synaptic transmission through activation of α1-adrenoceptors located on the soma of glycinergic interneurons.

CONCLUSIONS

Further investigation is required to clarify whether the RVL pacemaker cells recorded *in vitro* are themselves responsible for the regular strychnine-sensitive PSPs. It seems unlikely that they correspond to the fast pacemaker bulbospinal cells recorded *in vivo* which are inhibited by baroreceptor activation, since only a sympathoexcitatory function has been attributed to such neurons.[3] Alternatively, it is possible that at least some of the RVL pacemaker neurons belong to a small population of neurons, not yet clearly identified, subserving a sympathoinhibitory function, such as the 12% of bulbospinal RVL neurons found in the rabbit[27] or the 25% of RVL neurons of the decerebrate rat[14] that were excited by baroreceptor inputs. To date, there is more than one piece of evidence supporting the existence of RVL neurons with pacemaker-like activity that act locally to provide tonic inhibition of sympathoexcitatory neurons or constitute an inhibitory link in the baroreceptor–vasomotor pathway. The present results underscore the strength and complexity of the inhibitory network in the RVL and suggest that it

could be a target for modulation by catecholaminergic agonists and centrally acting antihypertensive drugs.

Acknowledgements—We are grateful to Dr F. Kato for his valuable discussions and Prof. R. Schlichter for his comments when revising the manuscript. This work was funded by a European Community Grant (ERBCHRXCT 940569), Direction des Recherches et Techniques (DRET 91-131), CNRS (URA 1446) and the Ministère de l'Enseignement Supérieur et de la Recherche (MESR, Univ. Louis Pasteur, Strasbourg). A. Hayar is supported by a predoctoral scholarship (CIES, Univ. Louis Pasteur) and Dr P. Piguet holds a Biopharma Research Associate fellowship on cardiovascular diseases.

REFERENCES

1. Aghajanian G. K. and Rasmussen K. (1989) Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. *Synapse* **3,** 331–338.
2. Allen A. M., Adams J. M. and Guyenet P. G. (1993) Role of the spinal cord in generating the 2- to 6-Hz rhythm in rat sympathetic outflow. *Am. J. Physiol.* **264,** R938–R945.
3. Allen A. M. and Guyenet P. G. (1993) α2-Adrenoceptor-mediated inhibition of bulbospinal barosensitive cells of rat rostral medulla. *Am. J. Physiol.* **265,** R1065–R1075.
4. Amano M. and Kubo T. (1993) Involvement of both GABA-A and GABA-B receptors in tonic inhibitory control of blood-pressure at the rostral ventrolateral medulla of the rat. *Naunyn-Schmiedeberg's Arch. Pharmac.* **348,** 146–153.
5. Barman S. M. and Gebber G. L. (1989) Basis for the naturally occurring activity of rostral ventrolateral medullary sympathoexcitatory neurons. *Prog. Brain. Res.* **81,** 117–129.
6. Blessing W. W. (1988) Depressor neurons in rabbit caudal medulla act via GABA receptors in rostral medulla. *Am. J. Physiol.* **254,** H686–H692.
7. Blessing W. W. and Willoughby J. O. (1987) Neurons in the area postrema are the only catecholamine-synthesizing neurons in medulla or pons with projections to the rostral ventrolateral medulla (C1 area) in the rabbit. *Brain Res.* **419,** 336–340.
8. Bousquet P., Feldman J. and Schwartz J. (1984) Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. *J. Pharmac. exp. Ther.* **230,** 232–236.
9. Coote J. H. and Lewis D. I. (1995) Bulbospinal catecholamine neurones and sympathetic pattern generation. *J. Physiol. Pharmac.* **43,** 251–271.
10. Cunningham E. T., Miselis R. R. and Sawchenko P. E. (1994) The relationship of efferent projections from the area postrema to vagal motor and brain stem catecholamine-containing cell groups—an axonal transport and immunohistochemical study in the rat. *Neuroscience* **58,** 635–648.
11. Czyzewska-Safran H., Jastrzebski Z., Remiszewska M. and Wutkiewicz M. (1991) Effect of clonidine on blood pressure and GABAergic mechanisms in spontaneously hypertensive rats. *Eur. J. Pharmac.* **198,** 115–120.
12. De Oliveira C. V. R., Assumpcao J. A., Confessor Y. Q., Covalan L. and Cravo S. L. (1995) Glycine release in the rat ventrolateral medulla during baroreceptor stimulation. *Soc. Neurosci. Abstr.* **21,** 890.
13. Ernsberger P., Giuliano R., Willette R. N. and Reis D. J. (1990) Role of imidazole receptors in the vasodepressor response to clonidine analogs in the rostral ventrolateral medulla. *J. Pharmac. exp. Ther.* **253,** 408–418.
14. Frugière A. and Barillot J.-C. (1994) The medullary rostral ventrolateral pressor region: an electrophysiological study in decerebrate rat. *Neurosci. Lett.* **68,** 191–194.
15. Gellman R. L. and Aghajanian G. K. (1993) Pyramidal cells in piriform cortex receive a convergence of inputs from monoamine activated GABAergic interneurons. *Brain Res.* **600,** 63–73.
16. Granata A. R., Numao Y., Kumada M. and Reis D. J. (1986) A1 noradrenergic neurons tonically inhibit sympathoexcitatory neurons of C1 area in rat brainstem. *Brain Res.* **377,** 127–146.
17. Grenhoff J., North R. A. and Johnson S. W. (1995) $Alpha_1$-adrenergic effects on dopamine neurons recorded intracellularly in the rat midbrain slice. *Eur. J. Neurosci.* **7,** 1707–1713.
18. Grudt T. J., Williams J. T. and Travagli R. A. (1995) Inhibition by 5-hydroxytryptamine and noradrenaline in substantia gelatinosa of guinea-pig spinal trigeminal nucleus. *J. Physiol.* **485,** 113–120.
19. Guertzenstein P. G. and Silver A. (1974) Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. *J. Physiol.* **242,** 489–503.
20. Guyenet P. G. (1990) Role of the ventral medulla oblongata in blood pressure regulation. In *Central Regulation of Autonomic Functions* (eds Loewy A. D. and Spyer K. M.), pp. 145–167. Oxford University Press, New York.
21. Hayar A., Piguet P. and Feltz P. (1996) GABA-induced responses in electrophysiologically characterized neurons within the rostro-ventrolateral medulla *in vitro*. *Brain Res.* **709,** 173–183.
22. Kangrga I. M. and Loewy A. D. (1995) Whole-cell recordings from visualized C1 adrenergic bulbospinal neurons: ionic mechanisms underlying vasomotor tone. *Brain Res.* **670,** 215–232.
23. Kapoor V., Nakahara D., Blood R. J. and Chalmers J. P. (1990) Preferential release of neuroactive amino acids from the ventrolateral medulla of the rat *in vivo* as measured by microdialysis. *Neuroscience* **37,** 187–191.
24. Kawasaki H. and Takasaki K. (1986) Central alpha-2 adrenoceptor mediated hypertensive response to clonidine in conscious, normotensive rats. *J. Pharmac. exp. Ther.* **236,** 810–818.
25. Lewis C. A. and Faber D. S. (1993) GABA responses and their partial occlusion by glycine in cultured rat medullary neurons. *Neuroscience* **52,** 83–96.
26. Lewis D. L. and Coote J. H. (1994) Mediation of baroreceptor inhibition of sympathetic nerve activity via both a brainstem and spinal site in rats. *J. Physiol.* **481,** 197–208.
27. Li Y.-W., Gieroba Z. J., McAllen R. M. and Blessing W. W. (1991) Neurons in rabbit caudal ventrolateral medulla inhibit bulbospinal barosensitive neurons in rostral medulla. *Am. J. Physiol.* **261,** R44–R51.
28. Li Y.-W. and Guyenet P. G. (1995) Neuronal excitation by angiotensin II in the rostral ventrolateral medulla of the rat *in vitro*. *Am. J. Physiol.* **268,** R272–R277.

28a. Lipski J., Kanjhan R., Kruszewska B. and Rong W. (1996) Properties of presympathetic neurones in the rostral ventrolateral medulla in the rat: an intracellular study "*in vivo*". *J. Physiol.* **490,** 729–744.

28b. Lipski J., Kanjhan R., Kruszewska B. and Smith M. (1995) Barosensitive neurons in the rostral ventrolateral medulla of the rat *in vivo*: morphological properties and relationship to C1 adrenergic neurons. *Neuroscience* **69,** 601–618.
29. Luque J. M., Nelson N. and Richards J. G. (1995) Cellular expression of glycine transporter 2 messenger RNA exclusively in rat hindbrain and spinal cord. *Neuroscience* **64,** 525–535.
30. Madison D. V. and Nicoll R. A. (1988) Norepinephrine decreases synaptic inhibition in the rat hippocampus. *Brain Res.* **442,** 131–138.
31. Malhotra V. and Sapru H. N. (1994) Decrease in blood pressure and heart rate (HR) elicited by injections of glutamate (Glu) into the locus coeruleus (LC) are mediated by α1-adrenergic receptors in the nucleus ambiguus/ventrolateral medullary depressor area (NA/VLDA). *Soc. Neurosci. Abstr.* **20,** 1181.
32. Malhotra V. K., Kachroo A. and Sapru H. N. (1993) Cardiac effects of injections of epinephrine into the spinal intermediolateral column. *Am. J. Physiol.* **265,** H633–H641.
33. Meeley M. P., Ruggiero D. A., Ishitsuka T. and Reis D. J. (1985) Intrinsic γ-aminobutyric acid in the nucleus of the solitary tract and the rostral ventrolateral medulla of the rat: an immunocytochemical and biochemical study. *Neurosci. Lett.* **58,** 83–89.
34. Milner T. A., Pickel V. M., Park D. H., Joh T. H. and Reis D. J. (1987) Phenylethanolamine *N*-methyltransferase-containing neurons in the rostral ventrolateral medulla of the rat. I. Normal ultrastructure. *Brain Res.* **411,** 28–45.
35. Miyazaki T., Coote J. H. and Dun N. J. (1989) Excitatory and inhibitory effects of epinephrine on neonatal rat sympathetic preganglionic neurons *in vitro*. *Brain Res.* **497,** 108–116.
36. Moriguchi A., Mikami H., Otsuka A., Katahira K., Kohara K. and Ogihara T. (1995) Amino acids in the medulla oblongata contribute to baroreflex modulation by angiotensin II. *Brain Res. Bull.* **36,** 85–89.
37. Pan Z. Z., Grudt T. J. and Williams J. T. (1994) α_1-Adrenoceptors in rat dorsal raphe neurons: regulation of two potassium conductances. *J. Physiol.* **478,** 437–447.
38. Paton J. F. R., Ramirez J.-M. and Richter D. W. (1994) Mechanisms of respiratory rhythm generation change profoundly during early life in mice and rats. *Neurosci. Lett.* **170,** 167–170.
39. Reis D. J., Colanov E. V., Ruggiero D. A. and Sun M.-K. (1994) Sympatho-excitatory neurons of the rostral ventrolateral medulla are oxygen sensors and essential elements in the tonic and reflex control of the systemic and cerebral circulations. *J. Hypertens. Suppl.* **12,** S159–S180.
40. Richerson G. B. and Messer C. (1995) Effect of composition of experimental solutions on neuronal survival during rat brain slicing. *Expl Neurol.* **131,** 133–143.
41. Rosin D. L., Zeng D., Stornetta R. L., Norton F. R., Riley T., Okusa M. D., Guyenet P. G. and Lynch K. R. (1993) Immunohistochemical localization of α2-adrenergic receptors in catecholaminergic and other brainstem neurons in the rat. *Neuroscience* **56,** 149–155.
42. Ruggiero D. A., Cravo S. L., Golanov E., Gomez R., Anwar M. and Reis D. J. (1994) Adrenergic and non-adrenergic spinal projections of a cardiovascular-active pressor area of medulla oblangata: quantitative topographic analysis. *Brain Res.* **663,** 107–120.
43. Ruggiero D. A., Meely M. P., Anwar M. and Reis D. J. (1985) Newly identified GABAergic neurons in regions of the ventrolateral medulla which regulate blood pressure. *Brain Res.* **339,** 171–177.
44. Somogyi P., Minson J. B., Morilak D., Llewellyn-Smith I., McIlhinney J. R. A. and Chalmers J. (1989) Evidence for an excitatory amino acid pathway in the brainstem and for its involvement in cardiovascular control. *Brain Res.* **496,** 401–407.
45. Sun M.-K. and Guyenet P. G. (1986) Effect of clonidine and γ-aminobutyric acid on the discharges of medullo-spinal sympathoexcitatory neurons in the rat. *Brain Res.* **368,** 1–17.
46. Sun M.-K. and Guyenet P. G. (1990) Excitation of rostral medullary pacemaker neurons with putative sympatho-excitatory function by cyclic AMP and β-adrenoceptor agonists '*in vitro*'. *Brain Res.* **511,** 30–40.
47. Sun M.-K. and Reis D. J. (1995) GABA-mediated inhibition of pacemaker neurons of rostral ventrolateral medulla by clonidine *in vitro*. *Eur. J. Pharmac.* **276,** 291–296.
48. Sun M.-K., Young B. S., Hackett J. T. and Guyenet P. G. (1988) Reticulospinal pacemaker neurons of the rat rostral ventrolateral medulla with putative sympathoexcitatory function: an intracellular study *in vitro*. *Brain Res.* **442,** 229–239.
49. Sved A. F. (1989) PNMT-containing catecholaminergic neurons are not necessarily adrenergic. *Brain Res.* **481,** 113–118.
50. Sved A. F. (1990) Effect of monoamine oxidase inhibition on catecholamine levels: evidence for synthesis but not storage of epinephrine in rat spinal cord. *Brain Res.* **512,** 253–258.
51. Triller A., Cluzeau F. and Korn H. (1987) Gamma-aminobutyric acid-containing terminals can be apposed to glycine receptors at central synapses. *J. Cell Biol.* **104,** 947–956.
52. White W. F., O'Gorman S. and Roe A. W. (1990) Three-dimensional autoradiographic localization of quench-corrected glycine receptor specific activity in the mouse brain using [^{3}H]-strychnine as the ligand. *J. Neurosci.* **10,** 795–813.
53. Yoshimura M., Polosa C. and Nishi S. (1989) Multiple actions of noradrenaline on sympathetic preganglionic neurons of the cat studied in the spinal cord slice. *Prog. Brain Res.* **81,** 181–190.
54. Zagon A. (1995) Internal connections in the rostral ventromedial medulla of the rat. *J. auton. nerv. Syst.* **53,** 43–56.
55. Zhong S., Huang Z.-S., Gebber G. L. and Barman S. M. (1993) Role of the brain stem in generating the 2- to 6-Hz oscillation in sympathetic nerve discharge. *Am. J. Physiol.* **265,** R1026–R1035.

(*Accepted* 23 *July* 1996)