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# Pharmacology of Reticulospinal Vasomotor Neurons in Cardiovascular Regulation

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## Introduction

### A. Arterial Pressure and Sympathetic Tone

Arterial pressure (AP), generated by the heart, which pumps blood into the closed circulatory system, and vascular tone are basic features of life in humans. They are controlled and greatly influenced by the sympathetic nervous system. Abnormality in sympathetic integration and control results in almost all types of cardiovascular diseases, including hypertension, hypotension, neurogenic cardiac arrhythmias, and ischemic stroke and is often associated with other diseases and complicates their treatments. Correcting such abnormality through the use of pharmacological agents has mobilized and will continue to mobilize investigators around the world. In addition, many compounds disturb cardiovascular functions either through their action on the heart and vessels or the nervous system that controls cardiovascular performance. The frequency and severity of such adverse effects often limit their potential values as therapeutic drugs.

The success of pharmacological therapies of cardiovascular diseases, including efforts to reduce adverse cardiovascular effects, depends on how much we know of cardiovascular mechanisms and their neural control. A fundamental understanding that has emerged is that reticulospinal vasomotor neurons, located in the rostroventrolateral reticular nucleus (RVL) of the medulla oblongata, function as an essential part of the life center in the medulla and play a critical role in the neural control of cardiovascular performance in health and disease. Activity of these RVL vasomotor neurons can be altered by applying a variety of substances (Sun et al., 1992b, c; Huangfu et al., 1995), of which many act either as endogenous transmitters or modulators or via mimicking or blocking synaptic transmission. Given the importance of these neurons in the regulation of sympathetic nervous outflow from the brain (see section I. C), their altered activity is bound to result in marked changes in cardiovascular performance. This article aims to provide an overview of current knowledge of this active field, focusing on the pharmacology of these RVL-spinal vasomotor neurons. The cellular and molecular mechanisms unraveled may provide the basis for the development of better therapeutic agents.

The understanding of the pharmacology of these vasomotor neurons, however, first requires some knowledge of the functions of these neurons and their relevance in the brainstem networks and cardiovascular control. Therefore, these are discussed briefly first. Reference will be made, in most instances, to more recent articles in the English language. Readers seeking further information of detailed neuronal mechanisms are referred to several recent reviews and monographs (Guyenet, 1990; Dampney, 1994a, b; Reis et al., 1994; Spyer, 1994; Sun and Reis, 1994b; Sun, 1995).

### B. Sympathetic "Premotor" Neurons

The importance of vasomotor neurons in RVL in cardiovascular regulation is indicated by their critical role in generating resting vasoconstricting tone and in relaying central commands to the sympathetic preganglionic neurons in the spinal cord. RVL contains many spinal cord-projecting neurons that innervate monosynaptically the sympathetic preganglionic neurons as well as interneurons and neurons in the dorsal horn of the spinal cord. The essentiality of sympathoexcitatory drive from supraspinal neural structures to the spinal cord for normal cardiovascular functions is emphasized by the fact that the sympathetic preganglionic neurons, especially the vasoconstrictor type, are generally intrinsically inactive and depend on the descending excitatory inputs for the maintenance of normal resting tone and AP. Clarification of this descending tone-generator, of the mechanisms responsible for the normal and abnormal tone, and of the potentials for pharmacological interventions therefore holds great interest. It should be mentioned, however, that RVL is not the only nucleus that projects to the intermediolateral cell column (IML) of the spinal cord, where the sympathetic preganglionic neurons are located; therefore, it is not the only group of sympathetic "premotor" neurons. Other "premotor" neurons include those in the medullary raphe nuclei, dorsal medulla oblongata, ventromedial medulla oblongata, the A5 area, the paraventricular, caudal lateral, perifornical hypothalamus, and midbrain periaqueductal gray. IML also receives inputs from the dorsal horn (laminae I, II, and IV) and the intermediate gray matter (lamina VII) of the spinal cord. Except for the RVL neurons, these "premotor" neurons, however, do not appear to play a significant role in the maintenance of resting sympathetic nerve activity (SNA) and AP, because, unlike RVL vasomotor neurons, their inhibition generally does not

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AMPA, α-amino-3-hydroxy-5-methyl-isoxazole-propionic acid; Ang, angiotensin; AP, arterial pressure; APV, DL-2-amino-5-phosphovaleric acid; ATP, adenosine 5′-triphosphate; CDS, clonidine-displacing substance; cGMP, cyclic guanosine monophosphate; CGRP, calcitonin gene-related peptide; CNS, central nervous system; CSF, cerebrospinal fluid; CVL, caudal ventrolateral medulla; DOCA, decorticosterone acetate; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; ECG, electrocardiogram; ET, endothelin; GABA, γ-aminobutyric acid; Glut, glutamate; HR, heart rate; 5-HT, 5-hydroxytryptamine; i.c., intracisternal; $IC_{50}$, concentration that inhibits 50%; i.c.v., intracerebroventricular; iGlutR, ionotropic glutamate receptor; IML, intermediolateral cell column; i.p., intraperitoneal; IR, immunoreactive; L-NMA, $N^G$-methyl-L-arginine; L-NNA, $N^G$-nitro-L-arginine; L-NAME, L-nitro-arginine-methyl-ester; i.v., intravenous; LC, locus coeruleus; mGlutR, metabotropic glutamate receptor; mRNA, messenger ribonucleic acid; NADPH, reduced nicotinamide adenine dinucleotide phosphate; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; NPY, neuropeptide Y; NTS, nucleus tractus solitarius; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propyl-amino)tetralin; PNMT, phenylethanolamine-N-methyltransferase; RVL, rostroventrolateral reticular nucleus; SHR, spontaneously hypertensive rat; SNA, sympathetic nerve activity; TRH, thyrotropin-releasing hormone; TTX, tetrodotoxin; WKY, Wistar-Kyoto (rats).

result in a dramatic decline in SNA and AP. However, assuming that the connections with the sympathetic preganglionic neurons suggest direct and/or indirect involvement in control of SNA, these neurons may play different roles in the control (or fine regulation) of different circulatory beds. Thus, RVL could be bypassed in some yet-to-be revealed forms of supraspinal sympathetic responses.

It should be indicated that the descending sympathetic vasomotor tone from RVL and the sympathetic nerve activity at large represent the neural mechanism that the body depends on for a rapid AP control and resting vasoconstrictor activity. A huge amount of evidence indicates that inhibition and/or lesions of RVL mimic cervical spinal transection, resulting in a rapid reduction in mean AP to 50 to 60 mmHg (for reviews, see Guyenet, 1990; Dampney, 1994a, b; Reis et al., 1994; Spyer, 1994; Sun and Reis, 1994b; Sun, 1995), if such lesions and inhibition are effective and precisely placed in RVL in the preparations in which their resting AP depends on an active SNA. A gradual normalization of AP within days after permanent lesions of RVL or cervical spinal transection does occur in the surviving animals and humans and is believed by some to be the evidence questioning the importance of sympathetic "premotor" RVL neurons and SNA in AP regulation. The question arises from a mix-up of two different concepts: (*a*) the contribution of sympathetic premotor activity to the maintenance of AP in intact animals and humans and (*b*) the readiness of other mechanisms to compensate for the AP decrease when SNA is suppressed. A significant reduction in AP may trigger several controller systems, depending on the severity and duration of the signal (Guyton, 1991). Thus, SNA is elevated for a rapid increase in AP. When speed is not primary, the hormonal system may also be called into play, especially when the sympathetic nervous system is inhibited or damaged. Within hours and days, a kidney pressure controller system also is induced that increases body fluid volume when AP decreases and, therefore, serves as a long-term pressure control. Mechanisms from these three pressure controller systems are known to compensate for one another, although with different speeds (Guyton, 1991). For instance, the kidney-fluid mechanism alone is capable of restoring and then maintaining AP at the "normal" level (Guyton, 1991) if the signal is severe enough to trigger the compensatory mechanisms and if the compensatory mechanisms are not inhibited. The threshold requirement to evoke the hormonal and kidney-fluid compensatory mechanisms when the sympathetic nervous system is inhibited is consistent with the observations that a unilateral chemical lesion of RVL results in permanently lowered AP in conscious, unrestrained dogs (Stith and Dormer, 1994) and that a unilateral microinjection of muscimol into RVL causes a lasting reduction in AP in decerebrate unanesthetized cats (Zhong et al., 1993), without evoking an obvious compensatory response. When the sympathetic nervous system is severely depressed or damaged (Cochrane and Nathan, 1989; Osborn et al., 1989), a gradual but full compensation of AP through the other controller systems in animals and humans means neither that the sympathetic nervous system is unimportant in normal AP control nor that the body can respond promptly and effectively to physiological and environmental demands for altering vascular circulation and cardiac output after a full compensation is established (Sun, 1995). Rapid responses (such as chemoreflex-mediated pressor responses), which depend on an activation of RVL-spinal vasomotor neurons and the sympathetic nervous system (Sun and Reis, 1996a), are lost when RVL neurons are inhibited, although AP is maintained at normal level by other means. Thus, one would not be surprised to observe small reductions in AP after placing lesions in RVL in the preparations in which resting AP is mainly maintained by the renin-angiotensin system and arginine vasopressin secretion (Cochrane et al., 1988; Cochrane and Nathan, 1993a, b; Cochrane and Nathan, 1994). Detailed discussion of the three controller systems and their interplay, however, is beyond this paper.

### *C. Rostroventrolateral Reticular Nucleus-Spinal Vasomotor Neurons*

RVL-spinal vasomotor neurons are located immediately caudal to the posterior end of the facial motor nucleus and ventral to the nucleus ambiguus complex, as the intermediate portion of the nucleus paragigantocellularis lateralis. Laterally, RVL extends to the trigeminal tract, caudally to the rostral edge of the lateral reticular nucleus, and medially to the gigantocellular reticular nucleus, the inferior olive, and the interfascicular hypoglossal nucleus. RVL in cats is sometimes referred to as the subretrofacial nucleus. According to Andrezik et al. (1981), this area in rats contains tens of thousands of neurons, differing in shapes of cell bodies and sizes (ranging from 30 to 150 $\mu m^2$ of cross-sectional area). The spinal cord-projecting RVL neurons, numbering in a few hundreds at most, represent a very small portion of the cells in RVL. It is not known how many of these neurons that project to the spinal cord function actually as vasomotor neurons. The coronal dendrites of RVL-spinal vasomotor neurons are substantially spread, including to the ventral surface (Sun et al., 1991). Their axons first travel in a dorsomedial direction before descending to the spinal cord (Ruggiero et al., 1989). These structural features may explain some pressor responses induced by electrical and chemical stimulation of RVL and its vicinity.

RVL-spinal neurons are heterogenous in terms of activity patterns and chemistry (Jansen et al., 1995; for reviews, see Dampney, 1994b; Sun, 1995). RVL contains a variety of neurons with different types of putative neurotransmitters and their biosynthetic enzymes, including phosphate-activated glutaminase (a glutamate-

synthesizing enzyme), phenylethanolamine-N-methyltransferase (PNMT) (an adrenaline-synthesizing enzyme), glutamic acid decarboxylase (a γ-aminobutyric acid (GABA)-synthesizing enzyme), reduced nicotinamide adenine dinucleotide phosphate (NADPH) diaphorase (a nitric oxide synthase (NOS), i.e., nitric oxide (NO) synthesizing enzyme), glycine, neuropeptide Y, substance P, angiotensin II, somatostatin, enkephalin, thyrotropin-releasing hormone, and probably oxytocin, vasoactive intestinal polypeptide, and cholecystokinin-8. They can be categorized on the basis of their intrinsic membrane properties into three elementary types. So far, the best-characterized RVL-spinal neurons are glutamatergic neurons (Guyenet, 1990; Morrison et al., 1988, 1991; Takayama and Miura, 1992; Miura et al., 1994) with intrinsic pacemaker activity (type I) in adults (Sun et al., 1988a, b, c, 1991), although their intrinsic rhythms are under significant modulation by synaptic inputs. The pacemaker currents of these neurons have been proposed to be of a gradual closing of outward potassium currents after each action potential upon a small persistent inward current (Sun, 1995).

RVL-spinal vasomotor neurons (fig. 1) project to the spinal cord, are barosensitive, and exhibit a constant firing rate (when counted as the number of spikes per second) at rest. Their projections to the spinal cord are tested and verified by the ability of their spontaneous spikes to collide in a critical period with the antidromic spikes, which are evoked by stimulating the axons and/or terminals through a stimulating electrode with its tip located near IML. Their discharges, like the vasoconstricting SNA (Sun and Guyenet, 1986b), show a strong cardiac rhythm (fig. 1). It remains to be determined whether some of them are PNMT-immunoreactive (IR) neurons. The PNMT-IR neurons are highly branched and have been shown to project to the spinal cord (Ross et al., 1984a), the locus coeruleus (LC), the parabrachial/Kölliker-Fuse complex, the hypothalamus, the midbrain periaqueductal gray, the paraventricular nucleus, the supraoptic nucleus (Haselton and Guyenet, 1990), and the nucleus raphe magnus (Tanaka et al., 1996). Differences have been noted as RVL vasomotor neurons with myelinated axons to the spinal cord in rats could not be antidromically stimulated from the perifornical hypothalamus in the limited number of cells tested (Sun and Guyenet, 1986c). Some PNMT-IR neurons, however, exhibit intrinsic pacemaker activity, especially when recorded in slices obtained from the neonates (Kangrga and Loewy, 1995; Li et al., 1995). The possibility that these "adrenergic" neurons may release L-glutamate (l-Glut) as their main transmitter has been proposed. Convincing evidence indicating their glutamatergic nature, however, has not been provided.

Evidence has been provided that blocking synaptic inputs onto RVL-spinal vasomotor neurons with receptor antagonists of Glut (Sun et al., 1988a; Gebber et al., 1989; but see Abrahams et al., 1994), the predominant excitatory neurotransmitter in the central nervous system (CNS), and with $CoCl_2$ and $MgCl_2$ (Trzebski and Baradziej, 1992) neither alters the low frequency dis-

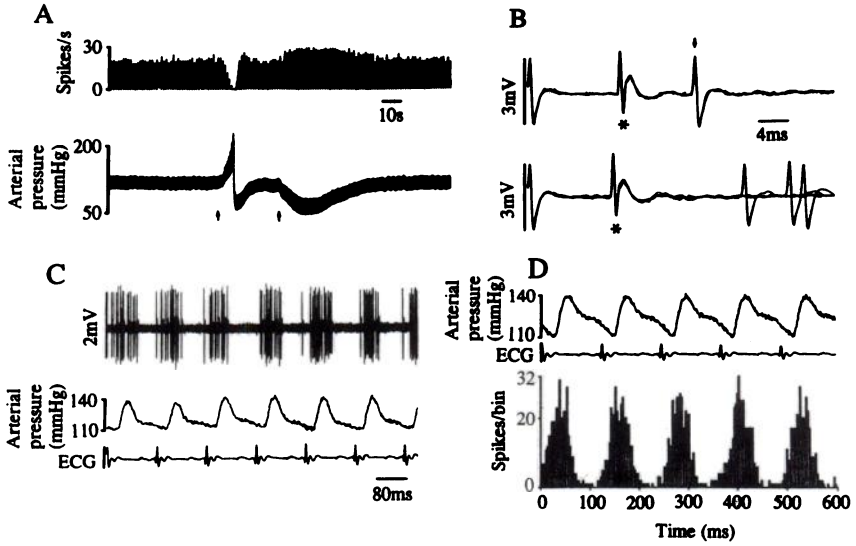


FIG. 1. Characterization of RVL-spinal vasomotor neurons. (A) Effect of change in arterial pressure on the neuronal discharge rate. Neuronal activity is displayed in the form of integrated rate histogram. Arterial pressure was elevated via descending aortic constriction (started at the first arrow and released when the neuron became silent) and reduced by i.v. injection of 10 μg sodium nitroprusside (at the second arrow). (B) Spinal cord projection of the neuron. The evoked antidromic spikes (arrow, top trace) by the spinal cord stimulation (asterisks) failed to occur at the recording site when the stimulation was applied within a critical period after spontaneously occurring spikes (bottom trace). (C) Pulse-synchronous discharge of an RVL-spinal vasomotor neuron. The arterial pressure trace (middle trace) and electrocardiogram (ECG) signal (bottom) represent a single sweep, whereas the trace of the neuronal discharge represents 12 consecutive sweeps, all triggered on ECG signals. (D) ECG-triggered time histograms of the neuronal activity (300 sweeps, 3 ms/bin). The top and middle traces represent averaged arterial pressure and ECG signals, respectively (50 sweeps each) (figure from Sun and Spyer, 1991b).

charge patterns of the sympathetic nerves nor reduces SNA and AP, indicating that intrinsic activity of RVL vasomotor neurons contribute to the descending sympathoexcitatory drive. This, however, does not rule out the possible contribution of synaptic inputs onto RVL neurons with intrinsic activity and of non-pacemaker RVL neurons to the resting SNA under some circumstances. The spinal cord-projecting neurons in RVL also include silent neurons (type II) and synaptically driven irregular firing neurons (type III; Granata, 1994; Lipski et al., 1995, 1996). Some of them may be sympathoinhibitory (Miura et al., 1994; Deuchars et al., 1995; Llewellyn-Smith et al., 1995). The spinal cord-projecting type III RVL neurons exhibit a resting discharge rate that varies (with no obvious causes) and have been shown to exhibit activity with no obvious cardiac rhythms (Granata, 1994). The lack of a cardiac rhythm in their firing probability, however, does not mean that they are baroinsensitive, for many non-cardiovascular RVL neurons, as defined by the lack of a cardiac cycle-related rhythm, have been shown to be inhibited (25%; Agarwal and Calaresu, 1992) and some (7%; Agarwal and Calaresu, 1992), activated by an increase in AP after intravenous (i. v.) L-phenylephrine. The majority of the physiological and pharmacological studies, as discussed in this review, have been performed on the type I RVL-spinal neurons. The spinal cord projection of the types II and III of RVL neurons indicates that these neurons may affect the activities of the sympathetic preganglionic neurons under some circumstances, but, otherwise, there are few clues to their functions and significance in sympathetic regulation. At present, available data do not distinguish whether the observed response is distinct for a particular class of neurons, such as the nonadrenergic or adrenergic neurons. Intracellular recording with markers to label the recorded neurons in vivo is possible but difficult, because long-term stable recordings are required for such study. Patch recording of visualized retrogradely labeled neurons in vitro (Kangrga and Loewy, 1995; Li et al., 1995) has definite advantage in comparing effects of pharmacologic agents on different types of neurons, because neurons can be marked individually and their chemical identities can be defined after the recordings. The shortcomings of such studies include the following: (*a*) the functional identity of the neurons is lost (location, projection, and types of transmitters do not necessarily define their vasomotor function; Sun, 1995); (*b*) neonatal animals are usually used for a clean cell surface, with unknown differences from adults; (*c*) the use of thin slices (120-250 $\mu$m) introduces the possibility of influence from injury currents; and (*d*) washing-out effects in the whole cell-recordings and its prevention may alter the responses. The in vitro response should be compared with those observed on the functionally identified vasomotor neurons in vivo.

The relevance and importance of RVL-spinal vasomotor neurons in cardiovascular control are three-fold. First, their inhibition or lesion virtually eliminates vasomotor SNA and reduces AP to the level seen in "spinal animals" (Willette et al., 1983, 1984b; Ross et al., 1984b; Sun and Reis, 1994c, 1996a), whereas synaptic blockade in RVL does not reduce much of SNA and AP (Trzebski and Baradziej, 1992). Second, a variety of reflex signals (Sun, 1995), such as of the arterial baroreflexes (Sun and Guyenet, 1985), chemoreflexes (Sun and Spyer, 1991d; Koshiya et al., 1993; Sun and Reis, 1995a; Koshiya and Guyenet, 1996), nociceptive sympatho-response (Sun and Spyer, 1991b) and centrally initiated sympathetic responses (Sun and Spyer, 1991a; Verberne, 1996), merge with them before descending to the spinal cord (fig. 2). Their involvement in the autonomic component associated with defense reaction has been proposed by Jansen and coworkers (Jansen et al., 1995). These neurons are, therefore, the final relay station in the medulla for a variety of sympathetic responses. Third, they sense chemical and physical changes in their environment and

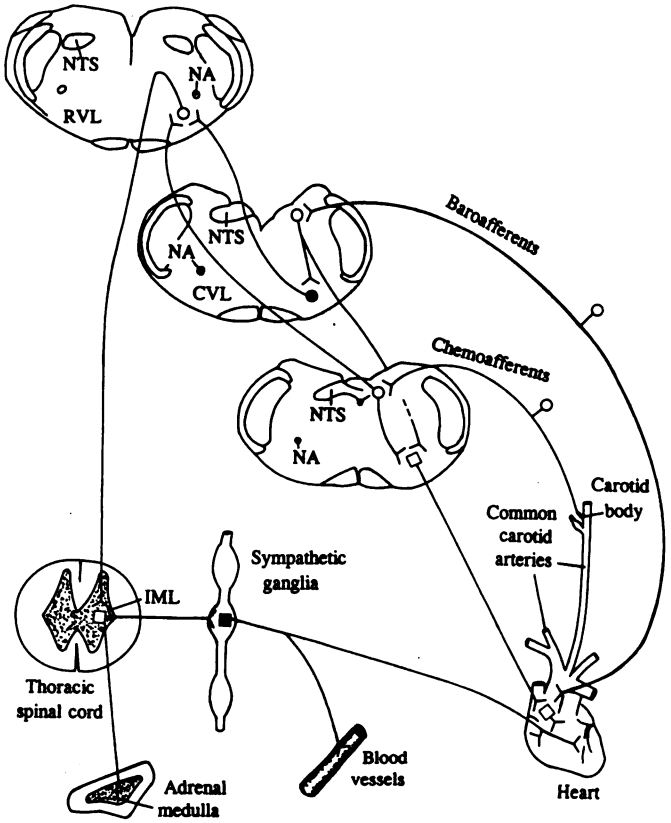


FIG. 2. Schematic drawing of RVL-spinal vasomotor neurons in medullary pathways that are responsible for baro- and chemoreflex regulation of sympathetic and cardiac vagal nerve activities in rats. Only one side is shown although most of these pathways are bilateral. Also omitted are the baroafferents from the carotid sinus, heart, and lung. In rats and rabbits, the aortic depressor nerves do not contain functional chemoafferents, whereas in cats, the nerves contain both the baroafferents and chemoafferets. Neurons are marked as either excitatory amino acidergic (○), GABAergic (●), cholinergic (□) or noradrenergic (■). NA: nucleus ambiguus.

initiate appropriate cardiovascular responses. Whether they and/or other neurons in RVL function as the essential element in the control of cerebrovascular circulation (Saeki et al., 1989; Reis et al., 1994; Underwood et al., 1994) remains to be determined. RVL vasomotor neurons are rapidly and selectively excited by local hypoxia (Sun et al., 1992a; Sun and Reis, 1993b, c, 1994d, e, 1995d, 1996a). Their hypoxic sensitivity plays an important role in adjusting cardiovascular performance according to the metabolic needs and status of oxygenation. It initiates a powerful sympathoexcitatory pressor response to protect vital organs, including the brain and heart, against hypoxic-ischemic damage and reduces hypoxic vasodilation of peripheral vascular beds, thus preserving tissues in the face of reductions in oxygen, which may occur as the result of environmental limitation or reduced tissue perfusion during hemorrhage, cardiac dysfunction, or ischemia. Their hypoxic sensitivity, however, may be involved in the generation and maintenance of several forms of cardiovascular diseases such as hypertension and neurogenic cardiac arrhythmias.

We now appreciate that RVL-spinal vasomotor neurons are central to the maintenance of resting vascular tone and control cardiovascular performance: their health is essential to normal cardiovascular functions, and their dysfunction can be a critical factor in pathogenesis of cardiovascular diseases. In spontaneously hypertensive rats (SHRs), arterial baroreflex inhibition of RVL vasomotor and sympathetic neurons, including thresholds, is shifted to a higher pressure without a dramatic change of the reflex gain, as defined by a brief AP increase, the natural stimulus (Sun and Guyenet, 1986b). The extreme importance of these precious vasomotor neurons in the control of cardiovascular performance and their great sensitivities to a variety of substances put them in the position as natural targets of neural commands in sympathetic control and as therapeutic targets in the treatments of cardiovascular diseases.

## II. Excitatory Amino Acids

### *A. Ionotropic Glutamate Receptors*

RVL-spinal vasomotor neurons are powerfully excited by L-Glut. The term L-glutamate is used in this review generically in the sense of excitatory amino acids. Evidence has been provided that demonstrates that microinjections of L-Glut into RVL increase SNA and AP and that iontophoresis of L-Glut onto singly recorded RVL-spinal vasomotor neurons rapidly excites them (Sun and Guyenet, 1986c). The receptors involved are of both N-methyl-D-aspartate (NMDA) and non-NMDA subtypes (Miura et al., 1991; Sun and Reis, 1995a) of the ligand-gated or ionotropic Glut receptor (iGlutR), because the neuronal and cardiovascular responses to L-Glut are eliminated by application of the iGlutR antagonist kynurenate in RVL (Sun and Guyenet, 1987; Sun and Reis, 1995a).

The Glut mechanism underlies several physiological inputs onto RVL vasomotor neurons, including excitatory inputs from the vagal afferents (Sun and Guyenet, 1987; Urbanski and Sapru, 1988), the amygdala (Takayama and Miura, 1991), the perifornical hypothalamus (Sun and Guyenet, 1986c) and the arterial chemoreceptors (Kubo et al., 1993; Amano et al., 1994; Sun and Reis, 1995a). Activation of arterial chemoreceptors rapidly excites RVL-spinal vasomotor neurons, resulting in a pressor response. Blocking glutamatergic transmission onto RVL vasomotor neurons with kynurenate abolishes the corresponding responses (Sun and Reis, 1995a). The receptors that mediate the chemoreflex excitation of RVL vasomotor neurons have been further characterized as the NMDA subtype because blocking the NMDA receptors with co-iontophoresis of the NMDA receptor antagonist DL-2-amino-5-phosphovaleric acid (APV) eliminates chemoreflex excitation of the recorded vasomotor neurons without affecting the discharge rate and excitatory responses of the neurons to kainate and quisqualate (Sun and Reis, 1995a). Microinjections of APV into RVL abolish (Kubo et al., 1993; Sun and Reis, 1995a)—but injections of the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione have no effect on—the chemoreflex-mediated pressor response (Kubo et al., 1993; Amano et al., 1994). These glutamatergic inputs do not appear very active under resting and anesthetized conditions, because, unlike the pre-inspiratory and expiratory neurons in RVL (Sun and Reis, 1996d), an effective blockade of both NMDA and non-NMDA iGlutR in RVL usually has no marked effect on their resting activity (Sun and Guyenet, 1986c; Sun and Reis, 1995a) and AP (Sun and Guyenet, 1986c, 1987; Urbanski and Sapru, 1988; Kubo et al., 1993; Amano et al., 1994; Lin et al., 1995; Sun and Reis, 1995a). However, a significant decrease in AP has been reported when non-NMDA iGlutR antagonists are injected into RVL in cats (Abrahams et al., 1994). It remains to be investigated whether the relative contribution of RVL pacemaker and non-pacemaker vasomotor neurons to the resting SNA differs in different species. Another possibility that might need to be considered is that local lesions or non-specific effects may be induced because of the focal volume and pressure changes during the penetration and microinjections. High concentrations of substances are often injected, e.g., for an effective blockade of receptors, and microinjections of the same volume of vehicle may not serve as a reliable control. Contrasting AP responses have been reported when receptor antagonists are introduced into RVL by microinjections or other means (intracisternal (i.c.) or intravenous (i.v.)): dramatic decreases in AP and SNA when microinjected versus no such decreases. The former result would naturally be taken as evidence indicating an essential and active role of the synaptic transmission in the mainte-

nance of resting AP, whereas the latter would indicate no active role. Such difference does not appear to result from a larger acting area or an insufficient blockade in the latter case. Such examples include kynurenate, several muscarinic antagonists, naloxone, and probably angiotensin II (Ang II) receptor antagonists.

### *B. Metabotropic Glutamate Receptors*

L-Glut may also serve as the endogenous agonist for the G-protein-linked or metabotropic Glut receptors (mGlutRs). Excitation of mGlutRs, which cannot be blocked by iGlutR antagonists (McDonald et al., 1993; Tizzano et al., 1993), in RVL has also been reported to evoke rapid cardiovascular responses (Tsuchihashi and Averill, 1993; Tsuchihashi et al., 1994). These results indicate that mGlutRs in RVL may participate in cardiovascular regulation but are contrary to the majority of the reports, which demonstrate that application of iGlutR antagonists, such as the wide-spectrum, competitive iGlutR antagonist kynurenate, effectively eliminates the excitatory response of RVL vasomotor neurons to iontophoresis of L-Glut (e.g., Sun and Guyenet, 1986c, 1987; Koshiya et al., 1993; Sun and Reis, 1995a). The acute studies, however, do not rule out the possibility that mGlutR activation may alter the functional state of RVL-spinal vasomotor neurons and produce a long-term effect. The involvement of mGlutR in cardiovascular regulation is further weakened by the general lack of effective blockade in RVL (Tsuchihashi and Averill, 1993; Tsuchihashi et al., 1994) by specific mGlutR antagonists, L-2-amino-3-phosphonopronate (Schoepp et al., 1990) or phenylglycine derivatives (Birse et al., 1993; Eaton et al., 1993), on the cardiovascular response evoked by mGlutR agonists.

The involvement of glutamatergic mechanisms in RVL in hypertension and as therapeutic targets in antihypertensive treatments will be discussed in XIII. A.

## III. Nitric Oxide

NO, a highly diffusible molecule that crosses cell membranes with a half-life of a few seconds, is produced by the enzyme NOS and has been implicated in a wide range of physiological and pharmacological actions in the cardiovascular, nervous and immune systems (Garthwaite et al., 1988; Dawson et al., 1992; Snyder, 1992; Montague et al., 1994), in addition to its involvement in the endothelium-dependent, cyclic guanosine monophosphate-mediated vaso-dilation (Doni et al., 1988). The constitutive and inducible (often induced as consequence of inflammation) NOS provides NO very rapidly and is localized throughout the CNS (Bredt et al., 1990; Förstermann et al., 1990; Sanders and Ward, 1992; Snyder, 1992; Calver et al., 1993; Persson, 1996).

The involvement of NO in cardiovascular regulation is suggested by the observation that acute, systemic application of NOS inhibitors results in an increase in AP (Sakuma et al., 1992; Cunha et al., 1993; Kumagai et al., 1993; Wang and Pang, 1993). There are reports that the pressor response involves, in addition to blocking the vasodilatory effect of NO, an increased SNA outflow, a response attenuated or abolished by transection of the cervical spinal cord (Sakuma et al., 1992). Chronic inhibition of NOS is also known to produce sustained hypertension, through the activation of the sympathetic nervous system and/or the renin-Ang system (Johnson and Freeman, 1992; Ribeiro et al., 1992; Manning et al., 1993; Morton et al., 1993; Zanchi et al., 1995). The increased SNA is either directly measured (Togashi et al., 1992) or indirectly assessed by the observation of increased plasma epinephrine and norepinephrine concentrations and greater reduction by systemic administration of phentolamine (Zanchi et al., 1995). The central NOS inhibition appears to be responsible for one-third to one-half of the raised AP after systemic administration of NOS inhibitors. Others have, however, reported no evidence of an increased sympathetic outflow (Hansen et al., 1994) or have reported that i.c. infusion of NOS blockers results in only a slight increase in AP and some increase in renal SNA (Togashi et al., 1992). The cause for such differences has not been clarified but may reflect different contribution of NO in cardiovascular regulation in the preparations.

NADPH diaphorase-positive neurons exist in RVL, and some of them have been found to project to the thoracic cord (Iadecola et al., 1993). RVL-spinal vasomotor neurons may be one of the targets for NO to influence SNA and AP. NO, released from nerve terminals, vessels supplying RVL area, and/or immunostimulated microglia and astrocytes, may affect these vasomotor neurons either as a neurotransmitter, a retrograde messenger in a Glut-releasing positive feedback system, or a modulator of the NMDA receptor channel complex of the neurons. NO may participate in neuronal response to iGlutR agonists because iGlutR agonists increase $[Ca^{2+}]_i$, which activates the $Ca^{2+}$/calmodulin-dependent NOS. NO stimulates Glut release, and Glut-induced NO formation may function as a positive feedback mechanism to enhance and/or maintain a sustained glutamatergic transmission. However, it has not been directly examined whether singly identified RVL-spinal vasomotor neurons are sensitive to NO and whether the NO system is active in RVL. The most relevant observation is that microinjections of NO donors into RVL inhibit SNA (Shapoval et al., 1991; Zanzinger et al., 1995a) and somato-sympathetic signal transmission through RVL without affecting the baroreflexes (Zanzinger et al., 1995a), suggesting that NO may inhibit RVL-spinal vasomotor neurons. An inhibitory action of NO on RVL vasomotor neurons is consistent with the evidence that microinjections of $N^G$-methyl-L-arginine (L-NMA) into RVL increase renal SNA and AP (Shapoval et al., 1991). In anesthetized barodenervated cats, L-nitro-arginine-methyl-ester (L-NAME)-induced profound AP increase is almost entirely abolished by cooling the ventral surface

of the rostral ventrolateral medulla (Zanzinger et al., 1994). However, this may indicate that the NOS inhibitor-induced increase in AP depends on signal transmission and/or vascular tone generated by the descending sympathoexcitatory drive, rather than a direct action of NOS inhibitors on RVL vasomotor neurons. Intracisternal administration of $N^G$-nitro-L-arginine (L-NNA) in anesthetized, chemo-denervated rats has been found to produce few changes in AP, SNA, and hypoxic pressor response (Sun and Reis, 1993b), suggesting either that NO system is not active in RVL or that its opposite effects on AP evoked in caudal ventrolateral medulla (CVL) and nucleus tractus solitarius (NTS) (Shapoval et al., 1991; Harada et al., 1993; but see Zanzinger et al., 1995b) tend to cancel each other.

It is now known that NMDA receptor channels have a redox-regulatory site that can affect NMDA receptor-mediated neuro-response and/or toxicity (Lei et al., 1992). Thus, reducing agents, such as dithiothreitol, enhance, and oxidizing agents, such as 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), a thiol-oxidizing agent (Gozlan et al., 1994), inhibit excitatory NMDA receptor responses (Lipton et al., 1996). NO, by supporting $NO^+$ transfer and S-nitrosylation of the thiol groups (cysteine residues; Sullivan et al., 1994) of NMDA receptor's redox modulatory site, is likely to produce a relatively persistent blockade of NMDA response (Lipton, 1993; Lipton et al., 1993; Lipton and Stamler, 1994). Such an action does not involve the formation of cGMP (Lei et al., 1992) and can be neuroprotective. This type of action, if produced, would block responses of RVL-spinal vasomotor neurons to chemoreflex excitation and attenuate defense-associated sympathoexcitation and stress-related hypertension. Direct evidence of such modulation of Glut-NMDA receptor transmission onto RVL-spinal vasomotor neurons and functional significance have not been provided. Interestingly, L-arginine has been reported to prevent the development of salt-sensitive hypertension in Dahl/Rapp rats, although not in SHRs (Chen and Sanders, 1991). Reaction of NO· with superoxide, on the other hand, leads to neurotoxicity through formation of peroxynitrite. The redox versatility of NO may offer a therapeutic avenue to harness neuroprotective action and avoid neurotoxicity by altering the ambient redox milieu (Lipton et al., 1993), which may be manipulatable through pharmacological means. The potential therapeutic values of manipulation of NO actions on RVL-spinal vasomotor neurons and synaptic inputs in some forms of hypertension require further investigation.

## IV. Acetylcholine

The cholinergic system in CNS is known to be involved in the regulation of cardiovascular functions, as activation of central cholinergic receptors or inhibition of cholinesterases induces cardiovascular responses, which vary from depressor to pressor and from bradycardia to tachycardia, depending on the species, the doses, the acting sites and the states of consciousness (Krstic and Djurkovic, 1973; Buccafusco and Brezenoff, 1979; De Wildt and Porsius, 1981; Brezenoff and Giuliano, 1982; Oktay et al., 1984; Hara et al., 1992; Ally et al., 1993; Özkutlu et al., 1993, 1995).

Stimulation of central muscarinic receptors in anesthetized rats results in increases in AP, mainly through an increase in SNA outflow to the vasculature (Brezenoff and Giuliano, 1982). The possible acting sites for acetylcholine (ACh) to evoke a pressor response have been proposed to include the amygdaloid complex (Özkutlu et al., 1995), posterior hypothalamic nucleus, AV3V region, hippocampus, LC, and RVL (Krstic and Djurkovic, 1973; Buccafusco and Brezenoff, 1979; Sundaram et al., 1988; Giuliano et al., 1989; De Luca et al., 1990; Natti and Li, 1990; Ohta et al., 1991; Hara et al., 1992; Haruta et al., 1992). Much of the AP effects elicited by interfering central cholinergic transmission, however, appears attributable to actions on RVL-spinal vasomotor neurons. RVL and its surrounding region contain cholinergic terminals, with the large majority (92%) of synaptic contacts on non-catechol-aminergic RVL neurons (Milner et al., 1989a) and a high density of muscarinic receptors (Cortes et al., 1984; Punnen et al., 1986; Deutch et al., 1987; Swanson et al., 1987; Ernsberger et al., 1988a; Giuliano et al., 1989; Arneric et al., 1990; Kinney et al., 1993). In urethane-anesthetized rats, i.v. administration of physostigmine, a carbamate acetylcholinesterase (AChE) inhibitor that is not highly charged at physiological pH, increases AP, heart rate (HR), and SNA (Giuliano et al., 1989). In anesthetized rats, the pressor response elicited by i.v. injections of physostigmine appears to be blocked by cervical spinal transection, but not by decerebration (Buccafusco, 1996). The responses are not mimicked by the quaternary AChE inhibitor neostigmine over a similar systemic dose range but can be blocked by microinjections into RVL of the $M_2$-cholinergic receptor antagonist AF-DX116 or the high-affinity choline uptake inhibitor hemicholinium-3. Neostigmine increases AP when introduced directly into CNS (Buccafusco, 1996). Iontophoresis of ACh onto singly identified RVL-spinal vasomotor neurons increases their activity in anesthetized rats, although the response is small (Sun and Guyenet, 1986c), probably because of the rapid hydrolysis of ACh and the effects of anesthetics, which are known to depress cholinergic responses of CNS neurons (Morin-Syrun et al., 1984; Foutz et al., 1987). Microinjections of cholinergic receptor agonists, or agents that release ACh from nerve terminals, into RVL have also been shown to increase AP (Willette et al., 1984b; Punnen et al., 1986; Sundaram and Sapru, 1988; Lee et al., 1991; Kubo et al., 1995a), through activating the muscarinic $M_2$ receptors (Sundaram et al., 1988; Giuliano et al., 1989). However, it is not clear whether the cholinergic inputs in RVL play a significant role in cardiovascular control at rest. A blockade of mus-

carinic receptors or inhibition of ACh synthesis within RVL has also been shown to decrease AP (Willette et al., 1984b; Punnen et al., 1986; Arneric et al., 1990; Lee et al., 1991) and an inhibition of AChE in RVL, increase AP (Kubo et al., 1995a), suggesting that endogenous ACh is tonically released in the area. This contrasts with the observation that i.c.v. administration of AF-DX116 (100 and 300 nmol) or methoctramine alone (10 and 30 nmol), both of which greatly inhibit i.v. physostigmine-induced elevations in AP, do not alter AP and HR (Özkutlu et al., 1993), suggesting that cholinergic transmission may normally contribute very little to the maintenance of resting SNA and AP. This raises the possibility that direct microinjections of the muscarinic antagonists into RVL may produce more than just muscarinic receptor blockade. As reviewed by Buccafusco (1996), muscarinic receptor antagonists such as atropine and scopolamine as used clinically generally do not lower AP (for review see Buccafusco, 1996). Thus, it has been proposed that central cholinergic neurons involved in AP regulation are not tonically active but are called into play to modulate SNA under specific physiological and pathophysiological conditions (Buccafusco, 1996). Elimination of abnormally enhanced cholinergic inputs onto RVL-spinal vasomotor neurons may have potential therapeutic values in some forms of hypertension (see XIII. A). Anyway, it would be interesting to examine whether blocking the cholinergic receptors of singly identified RVL-spinal vasomotor neurons dramatically changes their resting activity and whether the effects of such blockade differ between the normotensives and hypertensives.

## V. Catecholamines and Imidazolines

### A. β-Adrenoceptors

β-Adrenoceptor antagonists, such as propranolol, timolol and sotalol, are effective antihypertensive agents in human and animal models (Takeda and Bunag, 1980; Langemeijer et al., 1987). Their effect involves an inhibition of SNA via a central action. The cellular and molecular mechanisms involved in the β-adrenoceptor antagonist-induced sympathoinhibition, however, have not been clarified. This is partly because of an involvement of a chronic action, which may not be relevant to the findings in acute experiments. RVL-spinal vasomotor neurons may be the major target for centrally acting β-adrenoceptor antagonists. Microinjections of catecholamines into RVL produce mainly sympathoinhibition. However, after blocking α-adrenoceptors, a pressor effect is revealed and can be antagonized by β-adrenoceptor antagonists (Granata et al., 1986; Privitera et al., 1988). RVL vasomotor neurons are excited by isoproterenol (10 μM), a β-adrenoceptor agonist, and tyramine (1 mM), which releases catecholamines from catecholaminergic neurons in vitro (Sun and Guyenet, 1990). This action is blocked by propranolol (10 μM), appears to be mediated by adenylate cyclase, and in most cases takes a few minutes for the increased discharge rate to become evident. However, it is not known whether RVL vasomotor neurons receive significant catecholaminergic inputs. It has not been established whether the vasomotor neurons are significantly excited physiologically by endogenous catecholaminergic inputs, whose active role in RVL is supported only by the evidence that microinjections of propranolol (0.25-2 nmol) into RVL produce a lasting reduction in mean AP (Privitera et al., 1988). The effect of tyramine on RVL neurons in vitro might be produced through diffusion from remote sites. The extent of synaptic transmission by endogenous catecholamines and its contribution to the antihypertensive effects of β-adrenoceptor antagonists depend on how much of the activity of these neurons relies on synaptic inputs through activation of the β-adrenoceptors. One major concern in these studies is the fact that the blockade of β-adrenoceptors by β-adrenoceptor antagonists develops very rapidly, but their antihypertensive effects are produced more slowly. A chronic study of the effects of β-adrenoceptor antagonists on RVL-spinal vasomotor neurons is required to define the underlying cellular and molecular mechanisms responsible for the antihypertensive action. Indirect mechanisms should also be evaluated. Increased GABAergic activity after propranolol treatment has been observed in SHRs (Remiszewska et al., 1992), suggesting that enhanced GABAergic transmission may be involved in β-adrenoceptor antagonist-induced antihypertensive action.

### B. α-Adrenoceptors / Imidazoline Receptors

*1. Sites of action.* Systemic or central administrations of clonidine or clonidine-like substances generally decrease AP via inhibiting SNA through a central action (Kobinger, 1978; Ernsberger et al., 1987; Szabo et al., 1993), although there are reports that i.c.v. clonidine (1-10 μg) produces a pressor response in SHRs and Wistar-Kyoto (WKY) rats (Kawasaki et al., 1992). The hypotensive effect results mainly from a direct and/or indirect inhibition of RVL-spinal vasomotor neurons, because these neurons are inhibited by clonidine, and blocking α-adrenoceptors/imidazoline receptors in RVL eliminates the hypotensive effect of systemic clonidine (Sun and Guyenet, 1986a; Punnen et al., 1987; Ernsberger et al., 1987, 1990; Gomez et al., 1991; Tibirica et al., 1991b; Allen and Guyenet, 1993; Ruffolo et al., 1993; Guyenet et al., 1994; Sun and Reis, 1995b).

*2. Neuronal types.* Hypotensive effects of clonidine have been proposed to depend on its actions on central catecholaminergic neurons and/or terminals. This has been challenged, however, by the observations that depletion of catecholamines by reserpine, the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine, and the neurotoxin 6-hydroxydopamine does not dramatically affect the cardiovascular effects of clonidine (Tingley and Arnerić, 1990). Effects of clonidine have been examined on

RVL-spinal barosensitive neurons with relatively fast (2-8 m/s)- and slow (0.5 m/s)-conducting axons. (Whether or not one of the groups corresponds to PNMT-IR neurons remains to be determined.) Pharmacological doses of clonidine administered i.v., i.c. or iontophoretically inhibit all the slow-conducting and a small portion of the fast-conducting RVL-spinal barosensitive neurons (Sun and Guyenet, 1986a; Haselton and Guyenet, 1989), although many RVL barosensitive neurons are not affected (Sun and Guyenet, 1986a; Haselton and Guyenet, 1989; Clement and McCall, 1991). In anesthetized cats, no recorded RVL vasomotor neurons can be inhibited by clonidine iontophoresis (Clement and McCall, 1991), suggesting a mechanism of presynaptic or multiple sites of action. The slow-conducting neurons in rats are silenced in general at a small dose of clonidine that produces a small decrease in AP. The greater sensitivity of the slow-conducting barosensitive RVL neurons to clonidine often is accepted as evidence of their involvement in the clonidine-induced hypotensive response. Caution, however, should be exercised in interpreting the correlation between neuronal responses and sympathetic inhibition. It is quite possible that this population of RVL barosensitive neurons is significantly inhibited at lower doses of clonidine that do not significantly affect AP. A greater sensitivity to clonidine would dissociate their responses from effects on AP. Increased doses of clonidine, however, hyperpolarize and inhibit all the vasomotor pacemaker neurons in RVL (Sun and Reis, 1995b), consistent with the evidence that in anesthetized cats, ≥30 μg/kg of clonidine (i. v.) is required to silence the sympathetic nerves (Clement and McCall, 1991).

The most likely explanation for the general "lack" of inhibitory response of RVL vasomotor neurons in cats (and rats) to a direct microiontophoretic application of clonidine appears to be that an indirect action is involved. The clonidine-induced inhibition of RVL vasomotor neurons results, at least partially, from a release of GABA, because clonidine-induced membrane hyperpolarization is attenuated by tetrodotoxin (TTX), bicuculline, or $Cl^-$ channel blockers (Sun and Reis, 1995b). Thus, the effect of iontophoresis of clonidine on singly identified RVL-spinal vasomotor neurons depends on the number of GABAergic terminals affected by the applied clonidine. The indirect action is consistent with the observations that clonidine releases GABA (Pittaluga and Raiteri, 1988; Maura et al., 1988; Pittaluga et al., 1991) and that its hypotensive effects depend on the GABAergic system (Marmo et al., 1987; Tingley and Arnerić, 1990; Czyżewska-Szafran et al., 1991; Jastrzębski et al., 1995). Co-administration of bicuculline (1 mg/kg, i. p.) with clonidine has been found to abolish the hypotensive responses to clonidine (Jastrzębski et al., 1995).

*3. Receptor types.* One of the critical questions that must be addressed is which receptors clonidine-like substances act on to produce antihypertensive responses. Like many clonidine-like substances, clonidine binds to both the $\alpha_2$-adrenoceptors (inhibition constant ($K_i$) in RVL: 28 nM) and imidazoline (I; $K_i$ in RVL: 1 nM) receptors (Ernsberger et al., 1987, 1990). The dispute concerns whether the hypotensive response can be attributable to an action of these agents on $\alpha_2$-adrenoceptors or I receptors in RVL.

It is believed by some that an action on the $\alpha_2$-adrenoceptors in RVL is sufficient to explain the clonidine-induced hypotension (Nosjean and Guyenet, 1991; Allen and Guyenet, 1993; Head et al., 1993; Hieble and Kolpak, 1993; Szabo et al., 1993; Sannajust and Head, 1994; Chan et al., 1996; Orer et al., 1996). The existence of the $\alpha_2$-adrenoceptors in RVL is well established (Unnerstall et al., 1984; Rosin et al., 1993; Guyenet et al., 1994), although the receptor presence may not necessarily predict function. For instance, despite the existence of $\alpha_{2A}$-adrenoceptors (Unnerstall et al., 1984; Guyenet et al., 1994) in raphe serotonergic neurons and in raphe neurons with activity correlated to the 10-Hz rhythm in SNA (Barman and Gebber, 1992; Zhong et al., 1993), microinjections of clonidine into the medullary raphe complex have no effect on the high- or low-frequency components in SNA and AP (Orer et al., 1996). The essential role of $\alpha_2$-adrenoceptors in clonidine-induced hypotension is indicated by the evidence that application of $\alpha_2$-adrenoceptor agonists into RVL produces a depressor response and of $\alpha_2$-adrenoceptor antagonists, antagonizes the clonidine-induced AP effect. Iontophoresis of α-methyl-norepinephrine, an agent with negligible affinity for I receptors ($K_i$: 89 μM; Ernsberger et al., 1987) but with similar affinities as clonidine (0.99 nM) for the $\alpha_2$-adrenoceptors, produces a similar degree of an inhibition of RVL vasomotor neurons as does clonidine (Allen and Guyenet, 1993). Intravenous administration of the selective $\alpha_2$-adrenoceptor antagonists (Harrison et al., 1991), rauwolscine, SKF-86466, piperoxan, yohimbine, and/or RX-821002 (methoxyidazoxan) has been found repeatedly to reverse or antagonize the entire sympathoinhibitory and hypotensive response of clonidine and/or rilmenidine (Allen and Guyenet, 1993; Hieble and Kolpak, 1993; Ruffolo et al., 1993; Guyenet et al., 1994; Urban et al., 1994; Szabo and Urban, 1995). Iontophoretic application of methoxyidazoxan onto RVL vasomotor neurons has also been found to completely antagonize systemic clonidine-induced inhibition of the neurons (Allen and Guyenet, 1993; Stornetta et al., 1995). These are further supported by the receptor characterization and effects of receptor mutation. Three subtypes of $\alpha_2$-adrenoceptors ($\alpha_{2A}$, $\alpha_{2B}$, and $\alpha_{2C}$) have been isolated. These subtypes share a high degree of structural similarity (50-60% identity). Gene targeting has been used to introduce a point mutation into the $\alpha_{2A}$-subtype in the mouse genome. In the mutant mice, the hypotensive response to $\alpha_2$-adrenoceptor agonists of imidazoline analogs is lost (MacMillan et al., 1996). Disrup-

tion of the $\alpha_{2B}$ and $\alpha_{2C}$-*adrenoceptor* gene is not effective (Link et al., 1996). These results indicate an essential role of RVL $\alpha_2$, or $\alpha_{2A}$ to be more specific, adrenoceptors in the hypotensive action of clonidine-like substances.

However, evidence supporting a critical role of I receptors in RVL in clonidine-induced hypotension is substantial (Timmermans and Van Zwieten, 1982; Bousquet et al., 1984; Ernsberger et al., 1990; Gomez et al., 1991; Kawasaki et al., 1992; Bousquet, 1995). The hypotensive effects of clonidine in RVL have been reported to be mimicked by substances with I structure but not by catecholamines, such as $\alpha$-methyl-norepinephrine, a selective $\alpha_2$-adrenoceptor agonist (Bousquet et al., 1981, 1984; Ernsberger et al., 1990). Furthermore, the ability of clonidine-like substances to lower AP was found to correlate with their affinities for the I receptors but not to $\alpha_2$-adrenoceptor affinities (Ernsberger et al., 1987, 1988b, 1990). Caution, however, should be used, as the affinities of different agents with undefined nature as agonists, antagonists, or partial agonists are usually not directly correlated to magnitudes of responses because their intrinsic efficacies may vary. Some evidence of the existence of non-$\alpha$-adrenoceptor binding structures recognizing clonidine-like agents (Parini et al., 1989; Wang et al., 1992; Greney et al., 1994a, b) has been provided. These I receptors were mainly classified in two major subtypes: $I_1$- and $I_2$-receptors, based on their high ($I_1$) and low ($I_2$) affinity for clonidine (Michel and Insel, 1989; Parini et al., 1989; Ernsberger, 1990; Tesson et al., 1991; Michel and Ernsberger, 1992; Reis et al., 1992; Regunathan and Reis, 1996). $I_1$-receptors have been reported to be expressed on the cell surface, located in RVL, kidney, bovine adrenal medulla and human platelets (Regunathan and Reis, 1996), and functionally may represent the site responsible for the hemodynamic effects of imidazolines (Bricca et al., 1989, 1993). $I_1$ receptors, as defined in bovine ventrolateral medulla, represent about 30% of the [$^3$H]*p*-aminoclonidine sites that persist in the presence of 10 $\mu$M norepinephrine and show high affinity for clonidine ($K_d$: 1-5 nM; Ernsberger et al., 1987; Regunathan and Reis, 1996), although such high affinity binding sites are not observed in human brainstem (where binding sites were reported to have affinity for clonidine with $K_d$ of about 100 nM; Bricca et al., 1994). $I_2$-receptors, on the other hand, are proposed to be widely distributed and localized to mitochondrial membranes (Tesson et al., 1991; Regunathan et al., 1993). Additional evidence includes the observation that the hypotensive actions of clonidine-like substances are antagonized by agents with I-receptor selectivity but not by selective $\alpha_2$-adrenoceptor antagonists, injected into RVL (Ernsberger et al., 1987, 1990; Gomez et al., 1991). Assuming that microinjections of the $\alpha_2$-adrenoceptor antagonist produce an effective blockade of the $\alpha_2$-adrenoceptors in RVL, the results would indicate that the action of clonidine on RVL $\alpha_2$-adrenoceptors is not part of its therapeutic action of antihypertension. One of the immediate benefits of these studies is the possibility that if the hypotensive effect of clonidine could indeed be attributed to I receptors and the sedative effect to $\alpha_2$-adrenoceptors in LC (Bousquet, 1995), then their central antihypertensive effects could be dissociated from their sedative effect (Feldman et al., 1990; Tibirica et al., 1991b; Suaud-Chagny et al., 1992; Sannajust and Head, 1994; Chan et al., 1996).

Thus, microinjections of $\alpha_2$-adrenoceptor antagonists into RVL have been reported to either eliminate or have no influence on clonidine-like-substance-induced hypotension. There is no satisfactory explanation for these contrasting results. Part of the difficulty in defining what type of receptors is involved in the clonidine-induced hypotension is the current unavailability of selective I receptor antagonists devoid of affinity for $\alpha_2$-adrenoceptors. Perhaps one should consider the possibility that $\alpha_2$-adrenoceptors may, like the monoamine oxidase (Olmos et al., 1993; see below), have structures that are not part of the binding sites for catecholamines but recognize I-like structures and the effects of such binding depend on the interaction of the receptors and the structures of the imidazolines. Or, the same receptor molecule may show a different binding preference, depending on its functional states and environments. Indeed, such a possibility is probably supported by the recent studies in mutant mice lacking a functional $\alpha_{2A}$-adrenoceptor because of a point mutation introduced by gene targeting, which substituted an asparagine for the aspartate residue at position 79 (MacMillan et al., 1996). In these mutant mice, the hypotensive response to $\alpha_{2A}$-adrenoceptor agonists, including those with an imidazoline structure, is lost, indicating an essential role of the $\alpha_{2A}$-adrenoceptor subtype in the central hypotensive response that has been proposed to be mediated by RVL $\alpha_2$-adrenoceptor and imidazoline receptors. It would be interesting to determine whether this point mutation into the $\alpha_{2A}$ "knocks out" their affinity to both types of ligands. Tibirica et al. (1988) have reported that there might be a state or species difference in the type of receptors affected by clonidine in producing a depressor response, as yohimbine attenuates the clonidine-induced antihypertensive response in SHRs but not the hypotension in WKY rats. Idazoxan, which has relatively high affinity for I receptors, reverses the clonidine-induced hypotension in WKY rats (Tibirica et al., 1991a) but not the clonidine-induced antihypertensive response in SHRs (Tibirica et al., 1992). The observations suggest that one needs to distinguish a hypotensive action in the normotensives from an antihypertensive response in the hypertensives, consistent with the evidence that centrally acting adrenoceptor agonists such as clonidine and methyldopa are more effective in producing an antihypertensive response than a hypotensive response (Buccafusco, 1996). Others, however, have reported that no such difference exists in the clonidine-induced hypotension in SHRs and WKY

rats (Kawasaki et al., 1992). What causes such different observations is unclear. It is interesting that resting AP and HR in the mice with the mutated $\alpha_{2A}$-adrenoceptor subtype (MacMillan et al., 1996) or in genetically engineered mice deficient in either $\alpha_{2B}$ or $\alpha_{2C}$ adrenoceptors (Link et al., 1996) do not differ from the wild-type mice, suggesting that the receptors may not be essential components of the neural circuitry regulating cardiovascular function. Mechanisms independent of the $\alpha_2$-adrenoceptors or compensatory changes are therefore sufficient to establish basal cardiovascular set points.

Characterization of I receptors and actions also runs into the problem that imidazolines and imidazoline (imidazole)-like structures are recognized by many other cellular components. These include monoamine oxidase (Olmos et al., 1993; Bousquet, 1995; Carpene et al., 1995; Tesson et al., 1995), histamine $H_2$ receptors (Karppanen et al., 1976), Ang II type 1 receptors (Chiu et al., 1988; Wong et al., 1988; Timmermans et al., 1993; Weinstock et al., 1994; Aiyar et al., 1995), ethanol binding sites in GABA receptor channel complex (Suzdak et al., 1986), $GABA_C$ receptors (Kusama et al., 1993; Qian and Dowling, 1994, 1995), the nicotinic-cholinergic receptor channel complex (Powis and Baker, 1986; Cull-Candy et al., 1988; Loring, 1990), the prosthetic haem group of NOS (Babbedge et al., 1993; McMillan and Masters 1993; Moore et al., 1993a, b; Klatt et al., 1994), and some $K_{ATP}$ channels (Weitzel et al., 1980; Sense et al., 1989). Pretreatment with the monoamine oxidase inhibitors for 14 days in rats has been found to down-regulate non-adrenoceptor [$^3$H]idazoxan binding sites in the brain (Olmos et al., 1993). Bindings of imidazolines to nicotinic receptor channel complex alter the gating properties of the channel by reducing the time constants of the burst length and the frequency of opening of the channels but not the single-channel conductance (Powis and Baker, 1986; Cull-Candy et al., 1988; Loring, 1990). Rat brain constitutive NOS has been shown to exhibit spectral binding constants, $K_s$, of 2.5 $\mu$M for L-arginine and L-NMA and of 160 $\mu$M for imidazole (with 75% total protein at 1 mM imidazole), respectively (McMillan and Masters, 1993). In the $\beta$-cells of pancreatic islets, imidazolines block $K_{ATP}$ channels and thereby produce hypoglycemic effect because of their structural similarity to sulfonylureas (Weitzel et al., 1980; Sense et al., 1989). In addition, many of these substances, such as 2-nitroimizadoles, are molecules that have high electron affinity and therefore are retained by mitochondria, especially in hypoxic-ischemic conditions (Rumsey et al., 1994), which are characterized by the lack of appropriate electron acceptor at the cytochrome oxidase reaction. Their retention by hypoxic cells is so dramatic that these substances have been proposed to be useful as markers of hypoxia (Rumsey et al., 1994). A possibility is that this binding to enzymes, receptor channel complex, and reduced components may alter neuronal functions and interfere with haem redox cycling and respiration. Inhibition of cellular respiration may lead to reduced neuronal activity. For instance, Macrae and Browne (1995) have used [$^{14}$C]2-deoxyglucose autoradiography, reflecting glucose metabolism/cellular respiration, to examine the brain structures involved in the hypotensive effects of rilmendinine, a substance proposed to have some I-receptor selectivity, and B-PH 933, a selective $\alpha_2$-adrenoceptor agonist, in SHRs. At doses at which these agents produce similar reductions in mean AP, rilmenidine reduces glucose use in RVL, CVL, IML of the thoracic spinal cord, area postrema, NTS, and cuneate nucleus, whereas B-PH 933 does not significantly influence glucose use in these structures (Macrae and Browne, 1995). In short, binding and functional studies do not necessarily define events evoked from specific activation or blockade of the imidazoline receptors, even if the involvement of adrenoceptors is ruled out.

*4. Endogenous clonidine-displacing substances.* The proposed distinct I receptors in RVL raises the possibility of the existence of non-catecholamine endogenous ligands, for example the endogenous clonidine-displacing substance (CDS). Lots of efforts have been made to identify the endogenous CDS (Atlas and Burstein, 1984a, b; Meeley et al., 1986; Ernsberger et al., 1988c; Li et al., 1994). Non-catecholamine non-peptide substances with molecular weight of 588 Da (Atlas and Burstein, 1984a, b) or 550 Da (Meeley et al., 1986; Ernsberger et al., 1988c) have been proposed to be the endogenous CDS. The latter substance has been shown to inhibit clonidine binding at sites in ventrolateral medulla and to have a 30-fold selectivity for imidazole over $\alpha_2$-adrenoceptors in RVL (Ernsberger et al., 1988c). Injections of the partially purified CDS into RVL change AP, although opposite responses, an increase or a decrease, have been observed by different groups (Bousquet et al., 1986, 1987; Meeley et al., 1986).

Recently, agmatine, decarboxylated arginine, which is an amine with molecular weight of 130, prevalent in plants, bacteria, and invertebrate (Tabor and Tabor, 1984), has been identified as synthesized and stored in mammalian brain and responsible for the clonidine-displacing property of the brain preparation with $IC_{50}$ values of 4 $\mu$M, 0.7 $\mu$M, and 1 $\mu$M for inhibiting appropriate agonist binding at the $\alpha_2$-adrenergic, $I_1$, and $I_2$ receptors, respectively (Li et al., 1994). Agmatine has been proposed to be synthesized in mammal cells by the enzyme arginine decarboxylase, which also utilizes ornithine as a substrate with $K_m$ of 25 $\mu$M and has been found to specifically associate or exist in mitochondrial membranes (Regunanthan and Reis, 1996). Agmatine binds to I receptors and $\alpha_2$-adrenoceptors (Li et al., 1994; Bylund, 1995) but has been reported to have no obvious influence on the functions of the $\alpha_2$-adrenoceptors (Pinthong et al., 1995a, b).

Agmatine does not effectively penetrate the blood-brain barrier. Its i.v. and i.c. administration elicits entirely different patterns of cardiovascular responses

(Sun et al., 1995; Szabo et al., 1995): a pressor response when applied i.c. and a depressor response when applied i.v. in anesthetized rats. Both require very large doses and are produced through mechanisms that differ from those of clonidine (Sun et al., 1995). It does not affect activity of RVL-spinal vasomotor neurons when applied at rather high doses. The mechanism of its vasodilator action, evoked at very high doses (Gao et al., 1995; Piletz et al., 1995; Sun et al., 1995) remains to be determined. Its widespread existence in different mammalian tissues as reported (Regunanthan and Reis, 1996) is rather interesting, and both its physiological importance and functional relevance in cardiovascular regulation need further evaluation.

### *C. Levodopa*

L-3,4-dihydroxyphenylalanine (L-DOPA)-IR neurons and nerve fibers exist in RVL, CVL, and NTS (Tison et al., 1989). Neurons and nerve fibers that are tyrosine hydroxylase-immunoreactive but L-aromatic amino acid decarboxylase-immuno-negative have been found in several regions in the brainstem (Misu and Goshima, 1993; Misu et al., 1995), suggesting the existence of neurons that contains L-DOPA as an end product, therefore differing from those containing noradrenaline and adrenaline. In anesthetized rats, microdialysis reveals TTX-sensitive and $Ca^{2+}$-dependent release of L-DOPA in RVL (Misu et al., 1995). The release is sensitive to pretreatment with $\alpha$-methyl-*p*-tyrosine (200 mg/kg, intraperitoneal (i.p.); Goshima et al., 1993), an inhibitor of tyrosine hydroxylase. Microinjections of L-DOPA (10-300 ng) into RVL have been reported to produce dose-dependent increase in AP (Yue et al., 1993; Misu et al., 1995) and the response is blocked by microinjections of L-DOPA methyl ester, a competitive L-DOPA antagonist, into RVL (Yue et al., 1993). Microinjections of L-DOPA methyl ester into RVL have also been found to produce hypotension and bradycardia (Goshima et al., 1993), suggesting an active mechanism of L-DOPA as a neurotransmitter or modulator in RVL.

The basal L-DOPA release in RVL has been found to be higher by 30% in SHRs than in WKY rats and microinjections of smaller doses of L-DOPA into RVL of SHRs have been reported to produce greater increases in AP than those in WKYs (Yue et al., 1995). The possibility exists that the effect of L-DOPA may be related to an activation of $\beta$-adrenoceptors, because some effects of L-DOPA in other areas have been reported to be antagonized non-competitively by $\beta$-adrenoceptor antagonists (Goshima et al., 1986; Misu et al., 1986) but competitively by L-DOPA methyl ester (through non-$\beta$-adrenoceptors, non-$D_2$ receptors; Goshima et al., 1991). The L-DOPA actions may include pre-synaptic $\beta$-adrenoceptor-mediated release of catecholamines and Glut. Micromolar to millimolar L-DOPA increases the release of Glut from rat striatal slices through an L-DOPA ester-sensitive action (Goshima et al., 1993). In conscious rats, perfusion of nanomolar L-DOPA (10-100 nM) through probes induces, concentration-dependently, $Ca^{2+}$-dependent and TTX-sensitive striatal Glut release (Okumura et al., 1995). Direct action of L-DOPA and its antagonism on singly identified RVL-spinal vasomotor neurons and therapeutic values of such actions have not been examined and evaluated in any species.

## VI. 5-Hydroxytryptamine

RVL contains a high density of 5-HT-IR nerve terminals (Nicholas and Hancock, 1988; Ruggiero et al., 1989). Microinjections of 5-HT into RVL have been shown to elicit a delayed, long-lasting but moderate decrease in AP (Lovick, 1989). The cardiovascular responses differ when different subtypes of 5-HT receptors in RVL are selectively activated.

Microinjection of selective 5-$HT_{1A}$ receptor agonists, such 8-OH-DPAT, into RVL decrease SNA and AP (Lovick, 1989; Dabiré et al., 1990; Nosjean and Guyenet, 1991; Helke et al., 1993), but no such responses are elicited when injected into NTS or the raphe nuclei. Intravenous administration of 8-OH-DPAT decreases activities of RVL vasomotor and sympathetic neurons and AP (Kubo et al., 1995b), probably through a direct action on RVL vasomotor neurons. A direct action on RVL vasomotor neurons is supported by the result that the renal sympatho-inhibitory response can still be induced after depleting central catecholamines and 5-hydroxytryptamine (5-HT) (i.v. 5 mg/kg reserpine or i.p. 300 mg/kg $\alpha$-methyl-*p*-tyrosine, 18 h before the experiments; Dabiré et al., 1990), indicating that the responses are evoked at least partly through non-catecholaminergic non-serotonergic neurons. Evidence has been provided that RVL vasomotor neurons are inhibited by iontophoresis of the agent onto them (Kubo et al., 1995b). Co-iontophoresis of spiperone, a selective 5-$HT_{1A}$ receptor antagonist, inhibits the response of RVL vasomotor neurons to 8-OH-DPAT without affecting their resting firing rate, indicating the receptors involved and inactive nature of the serotonergic inputs onto RVL vasomotor neurons at rest. The latter is consistent with the observation that depletion of 5-HT (> 95%) with the serotonergic neurotoxin 5,7-dihydroxytryptamine (200 $\mu$g, i.c., 14-17 days before) alters neither the baseline mean AP nor HR values in rats (Helke et al., 1993). Microinjections of 5-$HT_{1A}$ antagonists into RVL have also been shown to inhibit the depressor response produced by systemic 8-OH-DPAT (Kubo et al., 1995b), indicating that inhibition of RVL vasomotor neurons is the main action of systemic 8-OH-DPAT in producing a depressor response. However, microinjections of idazoxan or rauwolscine into RVL have also been reported to attenuate sympathoinhibitory effect of i.v. 8-OH-DPAT (Nosjean and Guyenet, 1991), suggesting an involvement of RVL $\alpha_2$-adrenoceptors in the response.

Intravenous administration of a 5-$HT_2$ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), on the

other hand, increases AP and SNA and excites RVL vasomotor neurons to a similar extent (Clement and McCall, 1990), indicating that 5-$HT_2$ agonist-induced sympathoexcitatory pressor response results from increased activity of RVL vasomotor neurons. Iontophoresis of DOI onto RVL vasomotor neurons, however, has failed to excite them (Clement and McCall, 1990), suggesting that the responsible 5-$HT_2$ receptors, although located in RVL (Mandal et al., 1990), are probably presynaptic to RVL vasomotor neurons.

### VII. γ-Aminobutyric Acid and Glycine

RVL-spinal vasomotor neurons are inhibited by GABA and glycine. Iontophoresis of GABA or glycine onto singly identified RVL-spinal vasomotor neurons reversibly inhibits them (Sun and Guyenet, 1985, 1986a). Bilateral microinjections of muscimol, a long-lasting $GABA_A$ receptor agonist, GABA, or glycine into RVL result in an immediate decrease in the SNA and AP. When sufficient doses are applied, sympathetic nerves become silent and AP drops to the level of "spinal animals" (mean AP: 50-60 mmHg).

GABAergic inputs onto RVL vasomotor neurons are essential to arterial baroreflexes (Sun and Guyenet, 1985; 1987), vagal reflexes (Sun and Guyenet, 1987), area postrema reflexes (Sun and Spyer, 1991a; Wilson and Bonham, 1994), prefrontal insular cortex reflexes (Sun, 1992), and nociceptive (Sun and Spyer, 1991b)-mediated depressor responses. Blocking GABAergic transmission through the $GABA_A$ receptors on these neurons is known to increase their activity, SNA and AP. For example, iontophoresis of bicuculline, a $GABA_A$ receptor antagonist, onto the neurons increases their discharge rate and eliminates their sensitivity to baroreflex inhibition (Sun and Guyenet, 1985, 1987; Sun and Spyer, 1991a). Bilateral application of bicuculline on the ventral surface (Yamada et al., 1984) or microinjection into RVL abolishes baroreflex inhibition and pulse-related modulation of SNA (Sun and Guyenet, 1987) and increases AP (Willette et al., 1983, 1984a; Kubo and Kihara, 1987; Sun and Guyenet, 1987; Smith and Barron, 1990b). There is also a tonic baroreflex-independent GABAergic inputs onto RVL-spinal vasomotor neurons because blocking the $GABA_A$ receptors at the cellular level results in increases in activities of RVL-spinal vasomotor neurons (Sun and Guyenet, 1985, 1987), SNA and AP that are bigger than those observed from just blocking the arterial baroreflexes (Kubo and Kihara, 1987; Dampney et al., 1988; McCall, 1988).

The $GABA_B$ receptor agonist baclofen has also been reported to inhibit RVL-spinal vasomotor neurons and respiratory neurons (Li and Guyenet, 1995a); responses that are blocked by $GABA_B$ receptor antagonists CGP-54626A, CGP-55845A, and 2-hydroxysaclofen, but not by bicuculline. Interestingly, Li and Guyenet (1995a) have found that the application of a $GABA_B$ receptor antagonist does not significantly affect the GABA inhibition of RVL vasomotor neurons. Microinjections of CGP-55845A bilaterally into RVL do not increase AP, in contrast to Amano and Kubo's report (1993). The functional role of the transmission based on an activation of the $GABA_B$ receptors remains to be established.

Functional abnormality of GABAergic transmission and structural damage of GABAergic neurons may underlie many forms of cardiovascular diseases, including hypertension, and are discussed in XIII. B.

So far, little is known of physiological functions of glycine receptors on RVL vasomotor neurons other than that the inhibitory action of glycine on RVL vasomotor neurons is strychnine-sensitive (Sun and Guyenet, 1985). However, glycinergic inputs, if they exist, are probably not active under anesthetized conditions because microinjections of a glycine receptor antagonist strychnine into RVL do not affect AP (Ross et al., 1984b; Amano and Kubo, 1993), and iontophoresis of strychnine onto RVL vasomotor neurons eliminates responses of the neurons to glycine and taurine but does not change their discharge rate (Sun and Guyenet, 1985).

### VIII. Morphine and Opioid Peptides

In anesthetized rats, i.v. administration of morphine (10-1000 μg/kg) induces a dose-dependent hypotensive response, which fails to occur after pithing the spinal cord (White et al., 1995). In these pithed rats, i.v. phenylephrine is infused to maintain high AP, but morphine is inactive at doses up to 10 mg/kg (White et al., 1995), indicating that morphine acts in CNS to produce hypotension.

The hypotensive response of systemic morphine seems to involve a decrease in SNA (White et al., 1995) through inhibiting the vasomotor neurons in RVL. Microinjections of morphine and enkephalin into RVL decrease AP and HR, responses abolished by naloxone (Punnen et al., 1984; Punnen and Sapru, 1986; Sapru et al., 1987). Morphine and Met-enkephalin inhibit all the RVL vasomotor pacemaker neurons examined in vitro (Sun and Guyenet, 1989); when applied in vivo, morphine (10 μg/h i.c.v.) attenuates RVL catecholamine oxidation current in vivo, a response abolished by naloxone (1 mg/kg i.v.) (Wang et al., 1995). Effects of morphine on neuronal activity have also been examined on 10 PNMT-IR neurons in RVL. Inhibition was observed on seven of these, with two excited at lower (2 mg/kg) and inhibited at higher (7 mg/kg) doses (Baraban et al., 1995). The remaining three were excited only by morphine administration (Baraban et al., 1995). The heterogenous nature of the response and the observation that the majority of the PNMT-IR neurons were silenced at a dose (1 mg/kg) of morphine that does not produce many changes in AP (Baraban et al., 1995) again raises the question of the importance of activity of these PNMT-IR neurons to the regulation of SNA and AP. Their contribution to the opiate-evoked depressor responses, therefore, remains to be evaluated.

The powerful depression of RVL-spinal vasomotor neurons by opiate-like substances are likely direct, but it has not been ruled out whether part of the effect might result from an interaction with excitatory amino acids and suppression of membrane depolarization induced by L-Glut, as defined in other neurons (Zieglgänsberger and Tulloch, 1979; Kuznetsov and Godukhin, 1985). In anesthetized rats, systemic morphine-induced depressor response is reduced by an i.v. pre-application of adenosine $A_1/A_2$ receptor antagonists (White et al., 1995), suggesting that the hypotensive response may be partly mediated by adenosine.

The possibility that opiate-like substances in RVL may play a functional role in cardiovascular regulation is supported by the existence of a high density of enkephalin-IR terminals (Ruggiero et al., 1989), from the medial and commissural nuclei of NTS (Morilak et al., 1989), and synaptic contacts in RVL (Milner et al., 1989b). An opioid-like peptide system is activated by trauma and in disease states such as endotoxic shock (Xu et al., 1992) and hemorrhagic shock, and the potential values of antagonizing their inhibitory actions on RVL-spinal vasomotor neurons in shock therapies are discussed in XIII. B.

There are conflicting reports, however, as to whether the opiate inputs onto RVL vasomotor neurons are active at rest and therefore whether these play a role in the regulation of sympathetic tone. In un-bled, conscious rabbits, microinjections of naloxone into RVL produce a gradual and lasting increase in AP (Morilak et al., 1990), suggesting that endogenous opioids exert a tonic inhibitory influence on RVL vasomotor neurons. Others, however, have found that i.v. injections of naloxone (5 mg/kg, 4-5 min before) block opiate receptors in the brainstem but have little effect on AP in un-bled, conscious rabbits (Ludbrook and Rutter, 1988), suggesting that opiate system in the brainstem is not actively involved in cardiovascular regulation at rest but becomes activated during severe hemorrhage.

In opioid-dependent subjects, a sudden stop in opioid use or an administration of an opioid receptor antagonist such as naloxone provokes a withdrawal syndrome characterized by sympathetic, somatic, and parasympathetic hyperactivity. During opioid withdrawal, peripheral SNA is augmented (Delle et al., 1990; Baraban et al., 1993) and neurons in RVL become hyperactive and/or show increased c-*fos* immunoreactivity (Stornetta et al., 1993). Interestingly, RVL neurons may be responsible for hyperactivity observed in other brain neurons. Electrolytic lesions of RVL in opioid-dependent rats attenuate hyperactivity of LC neurons during opioid withdrawal (Rasmussen and Aghajanian, 1989). RVL neurons, most likely glutamatergic (Rasmussen and Aghajanian, 1989), project to LC and other higher centers, but it is not established whether such hyperactivity of LC neurons results from a direct hyperactive input from RVL. Acutely raised SNA may trigger stroke and cardiac arrhythmias. Such RVL hyper-activity as determined by measuring catecholamine oxidation current can be reduced by applying (i.c.v.) NMDA receptor antagonists and therefore is proposed to depend, in part, upon an activation of NMDA receptors (Wang et al., 1995). The immediate pressure response evoked by naloxone, however, precedes the increase in the catecholamine oxidation current by more than 15 min (Wang et al., 1995), suggesting that either the intensity of the catecholamine oxidation current does not represent the activity of the adrenergic neurons or the pressor response does not result from changed activity of these catecholaminergic neurons. It remains to be determined whether the hyperactivity of RVL vasomotor neurons (and therefore the increased AP and SNA) during opioid withdrawal can be abolished by blocking NMDA receptors of RVL-spinal vasomotor neurons. If so, elimination of the hyperactive glutamatergic inputs onto RVL vasomotor neurons or inhibition of the vasomotor neurons with inhibitory substances should eliminate the sympathetic hyperactivity and related cardiovascular risks associated with acute opioid withdrawal.

## IX. Other Neuropeptides

### *A. Vasopressin*

RVL vasomotor neurons are innervated by vasopressin-IR afferents originating from the paraventricular hypothalamic nucleus (Nilaver et al., 1980; Gomez et al., 1993) and dose-dependently (10-1,000 nM) excited by vasopressin (Sun and Guyenet, 1989). The effect is produced by an activation of the $V_1$ receptors because it is mimicked by the $V_1$ agonist and blocked by the $V_1$ antagonist but neither mimicked by the $V_2$ agonist nor blocked by $V_2$ antagonist (Sun and Guyenet, 1989). Application of vasopressin into RVL increases AP (Andreatta-van Luyen et al., 1990). Microinjections of vasopressin antagonists into RVL have been found to elicit no changes in AP. In urethane-anesthetized rats, i.c.v. microinjections of a $V_1$ receptor antagonist have also been reported to cause no changes in AP at rest, suggesting that vasopressinergic inputs and $V_1$ receptors in RVL are not tonically active in the control of cardiovascular functions. The inputs are, however, activated during hemorrhage to evoke a compensatory pressor response through sympathoexcitation because microinjections of vasopressin antagonists into RVL result in a fall in AP during early hemorrhage (Gomez et al., 1993). Intracerebroventricular administration of a $V_2$ antagonist, on the other hand, causes a transient augmentation of HR and AP (Xiao and Zhou, 1993), probably independent of RVL vasomotor neurons.

### *B. Angiotensin II*

Ang II is an octapeptide, whose effects on vascular tone as a vasoconstricting agent and on the release of arginine vasopressin and role in the renal renin-Ang

system have long been known (Hackenthal et al., 1990). The importance of renin-Ang system in some forms of essential hypertension is well established (Kim et al., 1995; Krege et al., 1995). With respect to RVL-spinal vasomotor neurons, although there is little doubt that augmented activities of RVL-spinal vasomotor neurons may contribute to hypertension related to higher levels of renin-Ang system and chronic Ang II-induced increases in AP, the exact role of these vasomotor neurons in acute Ang II-induced increases in AP remains disputed. On one hand, the Ang II receptors have been found to exist in RVL (Allen et al., 1988; Mendelsohn et al., 1988). Microinjections of Ang II into RVL increase AP and SNA (Allen et al., 1988; Sasaki and Dampney, 1990; Muratani et al., 1991; Li et al., 1992). Opposite cardiovascular effects are also elicited after microinjections of a specific antagonist of Ang II receptors into RVL (Sasaki and Dampney, 1990), suggesting a tonic activity of Ang II release in RVL. The central responses at an effective pressor dose may be mediated by catecholaminergic neurons located in the medulla. Administration of Ang II into the fourth ventricle in conscious rabbits induces a significant increase in the number of Fos-IR neurons in the ventrolateral medulla and NTS, with 50 to 75% of the Ang II-induced Fos-IR neurons in the ventrolateral medulla showing tyrosine hydroxylase immunoreactivity (Hirooka et al., 1996). On the other hand, evaluation of direct effects of Ang II on RVL vasomotor neurons in vivo runs into the problem that Ang II may induce an powerful hypoxic-ischemic pressor response, for Ang II is a potent vasoconstrictor agent and RVL vasomotor neurons respond to hypoxia-ischemia with great excitation. In general, however, RVL "cardiovascular" neurons are not consistently excited by iontophoretically applied Ang II (Chan et al., 1991). Their response, when observed, tends to be small in normotensive animals. Majority of such recorded neurons do not respond to iontophoresis of Ang II (Chan et al., 1991). In anesthetized rats, i.c. administration of Ang II, which produces a marked increase in AP, does not increase activities of RVL-spinal vasomotor neurons and renal and lumbar chain SNA (Sun and Reis, 1996b). The AP increase appears to result from the release of vasopressin into the peripheral circulation. because the AP response is largely attenuated by systemic administration of a vasopressin receptor antagonist. RVL-spinal vasomotor neurons are in fact inhibited because of the evoked AP increase, which activates the arterial baroreceptors. Unloading of the arterial baroreceptors by i.v. injections of nitroprusside to reduce AP to below their threshold does not reveal an increased basal firing rate. In conscious dogs, injections of Ang II into the third cerebral ventricle have been found to produce an immediate but lasting increase in AP, responses prevented by the administration of a vasopressin $V_1$ receptor antagonist (Łoń et al., 1996). The increase in AP is not associated with any immediate changes in plasma catecholamine concentrations after Ang II administration, but an increase is observed 30 min later (Łoń et al., 1996). Activation of the sympathetic nervous system therefore does not appear to be responsible for the increases in AP during the acute responses. One question that remains in these studies is whether a significant amount of applied Ang II actually leaks from the brain into the periphery to produce the cardiovascular response. In anesthetized barodenervated cats, cooling of the surface of the rostral ventrolateral medulla does not reduce the increase in AP, whereas the increase in AP that depends on RVL vasomotor tone is greatly attenuated (Zanzinger et al., 1994). In addition, central administration of Ang II monoclonal antibody (Moriguchi et al., 1995), antisense oligodeoxynucleotides specific for angiotensinogen messenger ribonucleic acid (mRNA), or Ang II receptor antagonists (Sun and Reis, 1996b) has minimal effect on AP in normotensive rats. Effectiveness of ganglionic blockers and adrenoceptor antagonists in reducing systemic Ang II-induced increase in AP has been taken as evidence of an involvement of SNA in the response but is likely to result from an elimination of the effects of Ang II in facilitating catecholamine release from adrenal chromaffin cells and sympathetic ganglia and in enhancing responses of vascular smooth muscle to catecholamines. These observations are consistent with the evidence that Brattleboro rats that do not synthesize vasopressin either show markedly reduced pressor response to central administration of Ang II (Haack and Möhring, 1978) or do not manifest any pressor response to intracranial administration of Ang II when they are not allowed to drink during the experimental session (Harland et al., 1988). In these rats, AP response to Ang II is dominant initially, with a vasoconstrictor response and later, with a neurogenic component in the chronic phase of Ang II-dependent hypertension (Cox and Bishop, 1991).

When examined in vitro, the pacemaker RVL neurons are not significantly excited by the application of $\leq 1$ $\mu$M Ang II (Sun and Guyenet, 1989). In thin slices obtained from neonatal rats, the effects of Ang II, most on C1 cells as well as on spinal cord-projecting neurons with an irregular non-bursting pattern of discharges, have been defined as a reduction in $K^+$ conductance (Li and Guyenet, 1995b, 1996). There is evidence, however, that spinal cord-projecting spontaneously active neurons in the RVL region with irregular non-bursting pattern of discharges are not cardiovascular because their activity does not show a cardiac rhythm (Granata, 1994). One problem with in vitro study is the fact that action of Ang II is not restricted only to vasomotor neurons. Ang II influences many neural functions, including dipsogenesis, modulation of hormone release from pituitary, interference with learning and motor activity, and regulation of body temperature and analgesia (Wright and Harding, 1994). The physiological and pharmacological meaning and significance of the action of Ang II on these

neurons in RVL remain to be studied. It should be noted that the lack of immediate effects of Ang II on identified RVL-spinal vasomotor neurons is consistent with the general contention that the Ang system plays a minimal role in AP control in normotensive animals (Gyurko et al., 1993; Timmermans et al., 1993) but does not rule out the possibility that the neurons might become sensitized in hypertensive species and to a long-term application of the peptide. These are discussed in XIII. A.

Ang II receptors have been divided into type 1 receptors, defined by their high affinity for Losartan, and type 2, by their high affinity for PD 123177. The type 1 or $AT_1$ receptors have been further subdivided into two subtypes, $AT_{1A}$ and $AT_{1B}$, which differ in their sequences and are coded by separate genes. Mice homozygous for a disruption of the gene coding for the $AT_{1A}$ receptor have virtually no pressor response to an i.v. injection of Ang II (Ito et al., 1995). Transgenic mice lacking the Ang II $AT_2$ receptors have normal AP but show an increased vasopressin response to Ang II administration (Hein et al., 1995). The functional importance of these subtypes in RVL has not been clarified.

### *C. Endothelins*

The involvement of endothelin (ET)-1, a potent vasoconstrictor and 21-amino-acid peptide originally isolated from vascular endothelial cells (Yanagisawa et al., 1988), in sympathetic regulation has been evaluated in several studies. Evidence suggests that ET-1 is biologically active in CNS. ET-1 is released from primary cultures of rat hypothalamic neurons (Krsmanovic et al., 1991). In rats, the concentration of ET-1 is higher in cerebrospinal fluid (CSF) (16 pM) than in the plasma (0.7-4.0 pM). The same is true in humans: 0.1-11 and 0.1-33 pM in CSF for ET-1 and ET-3, respectively, versus 0.4-1.6 and about 0.2 pM in the plasma for ET-1 and ET-3, respectively (Kuwaki et al., 1995 for review). Interestingly, ET-1 levels in CSF are reduced by about two-thirds when a sustained increase in AP is elicited by i.v. phenylephrine in rats, a response abolished by sinoaortic denervation (Mosqueda-Garcia et al., 1993), suggesting an baroreflex-mediated release. Nothing is known, however, of the neural circuit and its components in the brain, responsible for the barosensitive ET release.

ET-1-containing neurons are distributed widely in CNS in mammals, including humans (Lee et al., 1990). Autoradiographic studies document the presence of ET-1 binding sites in brain nuclei that are involved in cardiovascular regulation, including the supraoptic and paraventricular nuclei of the hypothalamus, NTS and RVL (Koseki et al., 1989). Intracisternal administration in rats causes either an immediate rise in renal SNA, HR, and AP (Kuwaki et al., 1990) or (20 pmol) hypotension and bradycardia, followed by hypertension (50 mmHg), bradycardia, and 100% mortality in urethane-anesthetized spontaneously breathing rats (Mosqueda-Garcia et al., 1995). The ET-induced cardiovascular responses are generally abolished by pre-treatment with BQ-123 (10 $\mu$g, i.c.v. 15 min early; Gulati et al., 1995). IRL 1620 (i.c.v.), a specific $ET_B$ receptor agonist, does not produce any changes in AP (Gulati et al., 1995), suggesting that the cardiovascular responses evoked by central administration of ET-1 are mediated by an action on $ET_A$ receptors. RVL represents the brainstem site where cardiovascular responses can be elicited at lowest doses of injected ET (Mosqueda-Garcia et al., 1993). RVL-spinal vasomotor neurons may therefore mediate the cardiovascular responses produced by central administration of ET, although its direct action on the singly identified vasomotor neurons has not been examined. Local application of ET-1 on the ventral surface of the medulla in rats causes an immediate rise in renal SNA, HR and AP (Kuwaki et al., 1990) and into RVL (1 pmol; Kuwaki et al., 1991a), an immediate rise in AP, bradycardia and respiratory depression, via an activation of the $ET_A$ receptors (Mosqueda-Garcia et al., 1995). The increases in AP and SNA last for only seconds and are rapidly reversed to decreases. Microinjections (1 pmol) of ET-1 into CVL, on the other hand, decrease renal SNA, AP, respiratory frequency, and phrenic nerve activity (Kuwaki et al., 1991a; Mosqueda-Garcia et al., 1995).

The physiological and pathophysiological significance of the sympathoexcitatory pressor response of RVL-spinal vasomotor neurons to ET, especially at large doses, remains to be evaluated. High rate of mortality resulting from respiratory impairment and cardiovascular collapse after its application into RVL or cisterna magna (Kuwaki et al., 1991b; Mosqueda-Garcia et al., 1993, 1995) indicates the pathological nature of the evoked responses. The responses, however, depend on many factors, including local ischemia caused by its potent vasoconstricting action and hypoxia caused by respiratory depression. The ischemic-hypoxic nature of the excitatory pressor responses of RVL-spinal vasomotor neurons to applied ET-1 is supported by the evidence that blocking $ET_A$ receptors in RVL attenuates the hypertension evoked by i.c. ET-1 and prevents mortality by 33%, whereas the initial hypotension appears to be mediated by CVL neurons because blocking $ET_A$ receptors in CVL inhibits the hypotensive response and reduces mortality by 25% (Mosqueda-Garcia et al., 1995). Thus, blocking $ET_A$ receptors in RVL vasomotor neurons and ET-1-induced synaptic inputs from CVL to RVL vasomotor neurons represents the major method in pharmacological therapy to prevent ET-induced acute collapse and death.

ET-1 has been postulated as a candidate gene for hypertension. In SHRs, ET-1 binding sites in RVL are down-regulated (Gulati and Rebello, 1991; van den Buuse and Itoh, 1993). However, administration of bosentan, a mixed $ET_A/ET_B$ receptor antagonist, has been found to have no effects on the development of

elevated AP in SHRs, unless the SHRs are treated with decorticosterone acetate (DOCA)-salt (Schiffrin et al., 1995). The DOCA-salt SHRs have abundant endothelial-1 mRNA in blood vessels and some sensitivity to bosentan treatment, but AP of bosentan-treated DOCA-salt SHRs is higher than that in age-matched untreated SHRs (Schiffrin et al., 1995). This is consistent with the observation that chronic treatment with bosentan reduces a portion of the elevated AP in DOCA-salt SHRs (Li et al., 1994), whereas bosentan treatment does not reduce AP in SHRs (Li and Schriffin, 1995). In the *ET-1* gene-knockout mice developed by Kurihara et al. (1994), striking effects on the development of the oropharyngeal region are observed in *ET-1* −/−homozygous mice, causing death at birth, probably from respiratory failure. The effects of ET-1 gene expression on AP are examined on *ET-1* +/− heterozygous mice, which appear normal and have ET-1 level in the plasma being 60% of the *ET-1* +/+ wild-type mice. AP is not reduced, but rather slightly increased by about 10 mmHg in these heterozygous mice. The underlying mechanisms are unclear. The possibility has been raised that the hypertension in the mutant heterozygous mice deficient in ET-1 may be related to diminished arterial $PO_2$ (90 versus 108 mmHg) and impaired cardiorespiratory control in response to hypoxia and hypercapnia, because of dysfunction of RVL neurons (Kuwaki et al., 1994, 1995).

### *D. Others*

Other neuropeptides that might directly excite RVL vasomotor neurons include substance P, neuropeptide Y, thyrotropin-releasing hormone (TRH), and calcitonin gene-related peptide (CGRP) (Sun and Guyenet, 1989). Microinjections of substance P or neuropeptide Y into RVL or their applications on the ventral surface increase AP (Tseng et al., 1988; Urbanski et al., 1989). Intracerebroventricular injections of CGRP have been shown to elevate AP and plasma norepinephrine in rats (Fisher et al., 1983). Terminals that are in RVL and immunoreactive for neuropeptide Y, substance P, TRH, and CGRP have been demonstrated (Hökfelt et al., 1975; Elskay et al., 1983; Skofitsch and Jocobowitz, 1985; Yamazoe et al., 1985; Milner et al., 1988; Nicholas and Hancock, 1988), although their functional meaning is unknown. TRH, however, may play a beneficial role in the course of recovery from hemorrhagic shock, which in fact increases brain TRH content, including the content in the medulla oblongata. Surviving rats have significantly more TRH in the medulla oblongata and other brain regions than do the non-surviving animals after hemorrhagic shock (Mizobe and Okuda, 1988). The therapeutical action and value of TRH on RVL-spinal vasomotor neurons are discussed in XIII. B.

Activation of kinin $B_2$ receptor in RVL has been found to increase AP to a significantly greater extent in SHRs than in WKY rats (Privitera et al., 1994; Privitera and Yates, 1995). Microinjections of the kinin $B_2$ receptor antagonist icatibant (Hoe 140) into RVL decrease AP more in SHR than in WKY rats (White et al., 1995), suggesting that local kinins in RVL may be active and involved in the maintenance of hypertension. Their effects and antagonism on activities of singly identified RVL-spinal vasomotor neurons, however, have not been investigated.

## X. Ethanol

Acute ethanol intoxication disturbs cardiovascular functions (Varga et al., 1994; Iwase et al., 1995) and increases risk of hemorrhagic stroke and death from heart disease (Beilin, 1995; Palmer et al., 1995). Part of its effects on cardiovascular function is produced by a mixed action of ethanol on inputs into RVL vasomotor neurons (Sun and Reis, 1992, 1996c), the very mechanisms the nervous system depends on for a rapid reflex control of cardiovascular performance. Ethanol enhances GABA-mediated inhibition of RVL-spinal vasomotor neurons (Sun and Reis, 1992) but depresses glutamatergic transmission (Sun and Reis, 1992), especially of those mediated by NMDA receptors in RVL (Mao and Abdel-Rahman, 1995; Sun and Reis, 1996c). In hippocampal pyramidal neurons, low ethanol concentrations (22-66 mM) enhance GABAergic inhibitory postsynaptic potentials only after blocking the $GABA_B$ receptors (Wan et al., 1996). The involvement of $GABA_B$ receptors in the ethanol effect on RVL vasomotor neurons has not been evaluated. Its action on GABA and NMDA receptors of brainstem cardiovascular neurons results in a net effect of depressing arterial baroreflexes (Mao and Abdel-Rahman, 1995) and chemoreflexes (Sun and Reis, 1996c).

Chronic ethanol intoxication is now recognized as one major contributor to prevalence and severity of hypertension (Varga et al., 1994; Iwase et al., 1995). Frequent episodes of disturbance of chemoreflexes and oxygenation may cause a delayed sympathetic excitation as the result of a direct and indirect response of RVL vasomotor neurons to hypoxia (Sun and Reis, 1996c) and lead to a sustainedly raised AP.

## XI. Adenosine and Adenosine 5′-Triphosphate

Adenosine is present in the brain at pharmacologically active levels (Barraco et al., 1987), and its concentrations increase further during hypoxia (Winn et al., 1981) and during increased neuronal activity (Lloyd et al., 1993). In vitro autoradiography has shown that the ventrolateral medulla in the rat brainstem has the highest density of adenosine $A_1$ receptors (St Lambert et al., 1996). Intravenous administration of adenosine in the range 10-3000 μg/kg in anesthetized rats produces a dose-dependent decrease in AP (White et al., 1995). Adenosine directly dilates vessels, but release of adenosine and activation of adenosine $A_1$ receptors in CNS have been proposed to partly mediate the morphine-induced hypotensive response (White et al., 1995). RVL

vasomotor neurons are sensitive to adenosine. In vitro, the corresponding intrinsically active RVL neurons are inhibited by adenosine (Sun and Guyenet, 1990), with an outward current under voltage-clamp recordings (Sun and Reis, 1994d). It has not been directly examined whether adenosine mediates morphine and enkephalin-induced inhibition of RVL-spinal vasomotor neurons. Although a brief period of hypoxia does not evoke an release of adenosine onto the RVL neurons, adenosine may be released during long-term ischemia-hypoxia, and released adenosine may inhibit RVL-spinal vasomotor neurons and be responsible for the failure of sympathetic drive at the late phase of severe hypoxia-ischemia. Efforts should be made to evaluate the potential and value of blocking the release of adenosine and adenosine receptors of RVL-spinal vasomotor neurons on preserving an active sympathetic tone and long-term recovery from hypoxia-ischemia and cardiac failure.

Adenosine 5′-triphosphate (ATP) has recently been identified as a fast neurotransmitter in both the central and peripheral nervous system, with its receptors in the cell plasma membrane or presynaptic nerve terminal (Sun and Stanley, 1996). RVL vasomotor neurons are rapidly excited by extracellular ATP and $\alpha$, $\beta$-methylene-ATP, a metabolically stable ATP analog (Sun et al., 1992c). Marked desensitization is followed after the initial excitation when $\alpha$, $\beta$-methylene-ATP is applied. Such a rapid excitatory response is not mimicked by adenosine (Sun et al., 1992c). It is not known, however, whether the responses are evoked by a direct action of ATP on the vasomotor neurons or indirectly through the release of neurotransmitters. The responses differ, however, from those to extracellular inositol hexakisphosphate (Sun et al., 1992b) because the effect of extracellular ATP on RVL vasomotor neurons is antagonized by suramin, a selective $P_2$-purinoceptor antagonist (Sun et al., 1992c), whereas the extracellular inositol hexakisphosphate effect on the vasomotor neurons results from its $Ca^{2+}$-chelating action (Sun et al., 1992b). Their therapeutic values in the treatment of hypotensive states, such as endotoxic shock, hemorrhagic shock, and the late phase of ischemia-hypoxia, stroke, and cardiac failure, remain to be examined.

## XII. Anesthetics

Anesthetics affect activities of RVL vasomotor neurons, SNA, and AP, depending on the types of anesthetics and the doses used. The types of action also underlie many CNS-acting drugs, such as antipsychotics, anxiolytics and antiepileptics, with sedative properties as adverse effects. In general, barbiturates tend to enhance GABAergic transmission and thereby potentiate such inputs and inhibit those neural components that are under tonic GABAergic control. In unanesthetized decerebrate rats, pentobarbital reduces activity of RVL-spinal vasomotor neurons and AP and enhances GABA-induced and baroreflex-mediated inhibition of these vasomotor neurons (Sun and Spyer, 1991c). Other general anesthetics, such as inhalation anesthetics and urethane, affect more of the glutamatergic transmission and responses, depending on the inputs. Thus, urethane attenuates response of RVL vasomotor neurons to local application of Glut and chemoreflex activation (Sun and Reis, 1995c). Deep anesthesia thereby suppresses the response to chemoreceptor activation and leaves a local vasodilation un-balanced when facing hypoxia-ischemia. Anesthetics affect not only ligand-gated but also voltage-gated ion channels (Franks and Lieb, 1994). Larger doses of anesthetics, in addition, will directly inhibit the neurons and abolish sympathetic tone.

Many of the cardiovascular and pharmacological responses, especially those depending on their actions in CNS, vary according to the state of the animals. Thus, i.c.v. administration of physostigmine in conscious rats produces a pressor response, which is significantly reduced in urethane-anesthetized animals (Özkutlu et al., 1995), indicating that, in addition to binding to multiple binding domains in the transmembrane region of ACh receptors (Eckenhoff, 1996), anesthesia depresses either ACh release from the nerve endings or cardiovascular reflexes.

## XIII. Clinical Relevance

Cardiovascular diseases have been the leading cause of death in developed countries for most of the Twentieth Century. Maintenance of normal AP and circulation through pharmacological means is and will remain one of the biggest challenges to modern medical practice, especially in clinical ills. Abnormalities in SNA and AP occur both ways. Sympathetic over-activity in most cases represents pathophysiological mechanisms or the primary cause of the cardiovascular abnormality, as in many cardiovascular disorders, such as cardiac failure, cerebral ischemia, stroke, a variety of forms of primary hypertension, and portal hypertension in hepatic cirrhosis, either reflexively or centrally initiated (Esler, 1995). On the other hand, impact of sympathetic suppression on health and recovery from a variety of diseases has been realized but rarely investigated. Low AP and SNA occur in the late phase of stroke, cerebral ischemia, trauma, and most of the severe ills, reflect the deteriorating conditions and greatly reduce the chance of recovery from the insults and permanent damage. The involvement of RVL-spinal vasomotor neurons in the sympathetic dysfunction (or knock-out), underlying mechanisms, and possible pharmacological means to restore SNA are worthy of intensive investigation. As for now, the discussion of clinical relevance of pharmacology of RVL-spinal vasomotor neurons will focus on hypertension, cardiac failure and shock.

### *A. Hypertension*

Over-activity of the sympathetic nervous system contributes significantly to primary hypertension and may

in fact underlie the pathology of many forms of hypertension, although what and how genetic defects result in the diseased states remain to be clarified (Lander and Schork, 1994; Smithies and Maeda, 1995). Abnormal integration and responses of RVL-spinal vasomotor neurons appear to play an important role in the generation and maintenance of hypertension. These may be specifically targeted in antihypertensive therapy.

*1. Enhanced glutamate receptor activation.* Enhanced glutamatergic inputs onto and/or increased sensitivity to Glut of RVL-spinal vasomotor neurons (Tsuchihashi et al., 1994) may contribute to the establishment and maintenance of hypertension, especially stress-related hypertension, and therefore represent a specific therapeutical target in the treatment of this disorder. RVL in SHRs has been found to receive more inputs from glutamatergic neurons in the lateral parabrachial nucleus and the Koelliker-Fuse nucleus but fewer glutamatergic inputs from the medial and commissure subnuclei of NTS than in WKY rats (Takayama and Miura, 1992). No such abnormal distribution of glutamatergic and GABAergic neurons that project to thoracic IML of the spinal cord in the brainstem has been found in SHRs (Miura et al., 1994). If the number corresponds to the levels of activity, enhanced activity is more likely to occur at the supraspinal level.

Not only does RVL in SHRs receive more glutamatergic inputs, but RVL vasomotor neurons also may be more sensitive to such inputs. Microinjections into RVL of anesthetized SHRs of (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid, a selective mGlutR agonist, and of L-Glut, NMDA or α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid, selective iGlutR agonists, all evoke significantly greater increases in AP and SNA than those in the age-matched WKY rats (Miura et al., 1991; Tsuchihashi et al., 1994; Lin et al., 1995; Yang et al., 1995). The question is whether the enhanced responses reflect damaged integration and reflex control in the hypertensive species or a specific abnormality related to L-Glut sensitivity in RVL. RVL-spinal vasomotor neurons in hypertensive animals generally show exaggerated cardiovascular responses (Chan et al., 1990) to stress, electrical stimulation, a variety of bioactive substances, and reflexively and centrally evoked pressor responses, probably reflecting impaired feedback control and/or altered response machinery. Others have, however, observed that pressor responses to L-Glut injection into RVL in SHRs do not differ from those in WKYs (Kubo et al., 1986; Smith and Barron, 1990a, b; Muratani et al., 1991, 1993; Kubo et al., 1995a). It therefore remains to be further examined whether RVL vasomotor neurons indeed show augmented sensitivity to L-Glut and whether increased glutamatergic inputs onto RVL-spinal vasomotor neurons are partly responsible for the initiation and maintenance of some forms of hypertension.

*2. Enhanced cholinergic transmission.* Central cholinergic neurons in general do not appear to be actively involved in AP regulation in normotensives (Buccafusco, 1996). Increased cholinergic transmission in RVL as an increase in cholinergic impulse flow has been proposed to play a role in the maintenance of hypertension in SHRs (Kubo et al., 1995a). Evidence is several-fold. First, i.v. injections of physostigmine, a centrally active AChE inhibitor, evoke a greater pressor response in SHRs as compared with that in WKY rats (Kubo and Tatsumi, 1979; Buccafusco and Spector, 1980; Makari et al., 1989), whereas producing a similar inhibition of AChE in RVL of SHR and WKY rats (Kubo et al., 1995a). Microinjections of atropine or scopolamine, muscarinic receptor antagonists, into RVL have also been found (Lee et al., 1991; Kubo et al., 1995a) to produce a greater depressor response in SHRs than in WKY rats. Furthermore, i.c.v. administration of hemicholinium-3, an ACh depeletor, produces significant reductions in AP in SHRs but little changes in AP in the normotensive WKY rats (Brezenoff and Caputi, 1980; Giuliano and Brezenoff, 1987), indicating that the cholinergic system in RVL is relatively inactive in the normotensives but more active in the hypertensives. Microdialysis also reveals a greater amount of ACh release in RVL of SHRs than of WKY rats, and the increased release in SHRs appears to be the result of a highly localized enhanced choline acetyltransferase activity and, therefore, ACh synthesis in RVL. The enhanced presynaptic mechanism is consistent with the evidence that the pressor response to ACh and carbachol injections into RVL does not differ between SHRs and WKY rats (Kubo et al., 1995a), indicating that postsynaptic events are not responsible for the observed alterations. Others have reported, however, that cardiovascular responses to microinjections of carbachol (1 nmol/site) into RVL are enhanced in SHRs (Lee et al., 1991). It is interesting that the portion of the enhanced ACh release in RVL of SHRs is TTX-sensitive but the basal amount of ACh release (6-7 pmol/20 min) is not (Kubo et al., 1995a). When examined in vitro, neither spontaneously nor $K^+$-evoked release of [$^3$H]ACh in RVL has been found to be altered in 12-16 week-old SHRs and WKY rats, suggesting that there is no abnormality in excitation-secretion coupling or ACh release per a nerve impulse in RVL cholinergic nerve terminals in SHRs.

It remains to be determined why cholinergic nerve impulses in RVL are enhanced in SHRs. Elimination of this abnormal cholinergic inputs in RVL would represent a potential intervention in antihypertensive therapy. This can been achieved either by blocking the inputs in RVL or by inhibiting the cholinergic neurons. It is, however, unclear where these cholinergic neurons are located and what triggers their enhanced tone. One of the possible sources of the cholinergic innervations is from the parabrachial and Kölliker-Fuse nuclei in the pons (Jones and Beaudet, 1987). These pontine neurons

projecting rostrally to the forebrain and caudally to the cervical spinal cord play an important role in the sleep-waking cycle (Lydic and Baghdoyan, 1989; Vanni-Mercier et al., 1989). Other cholinergic inputs are from the dorsolateral tegmental nucleus, the spinal cord, and the nucleus ambiguus (Milner et al., 1989a). Their particular role in the enhanced cholinergic inputs onto RVL-spinal vasomotor neurons has not been determined.

*3. Adrenoceptor agonists/antagonists.* β-Adrenoceptor antagonists and $\alpha_2$-adrenoceptor agonists are proved clinically effective antihypertensive agents. Their antihypertensive effects are produced mainly through an action on RVL-spinal vasomotor neurons, as discussed in section V. What is interesting is that despite their effectiveness in inhibiting RVL vasomotor neurons, no evidence suggests that abnormal adrenergic transmission is related to the generation and maintenance of hypertension. Thus, their effectiveness in antihypertension appears to rely on a direct and indirect inhibition of these vasomotor neurons. In fact, disruption of the genes encoding the $\alpha_2$-adrenoceptors in mice does not affect the set point of resting AP and HR (Link et al., 1996; MacMillan et al., 1996), suggesting that abnormal $\alpha_2$-adrenoceptors and their mediated synaptic transmission are not sufficient to result in the hypertensive state.

*4. Angiotensin II.* Brain Ang II appears to be involved in the pressure control in transgenic m*Ren*-2$^d$ hypertensive rats, although not in the normotensive rats (Moriguchi et al., 1995). In conscious rabbits, intravertebral infusion of Ang II resets the operating point of the HR baroreflex to a higher AP (Nishida et al., 1995), reduces the renal SNA (baroreflexive), but has no effect on the renal SNA baroreflex curve (Nishida et al., 1995). HR baroreflex resetting has been shown to be independent of the elevated AP (Brooks et al., 1993) but dependent on $\alpha_1$-adrenoceptors in the medulla (Nishda et al., 1995). Resetting of the sympathetic baroreflex does occur but requires several days of Ang II infusion (Bishop et al., 1995), whereas HR baroreflex resetting occurs within minutes of the administration of Ang II (Bishop et al., 1995). Whether sympathetic baroreflex resetting involves sensitization of RVL vasomotor neurons to Ang II remains to be determined, but the type of baroreflex resetting has been observed in RVL vasomotor neurons of SHRs and may underlie increased renal SNA in congestive heart failure (Ferguson, 1993; DiBona et al., 1995). The duration required for Ang II to reset the sympathetic baroreflex may suggest an involvement of altered gene expression and/or structures. It is therefore interesting and important to know what molecule(s) and/or structural changes are responsible for the resetting and how to reverse and/or terminate the process.

Increased sensitivity to Ang II of and/or Ang II inputs onto RVL-spinal vasomotor neurons in SHRs may be responsible for the hypertensive state. RVL vasomotor neurons in SHRs indeed show a greater excitatory response to iontophoresis of Ang II (Chan et al., 1991). Microinjections of a specific Ang II receptor antagonist into RVL have also been found to evoke greater magnitudes of depressor response in SHRs than in WKY rats (Muratani et al., 1993). Such microinjections into CVL also increase AP and SNA and modulate arterial baroreflexes (Sesoko et al., 1995), with greater response in SHRs than in WKY rats (Muratani et al., 1993). However, others have reported that microinjections of Ang II into RVL produce a small but similar increase in AP in SHRs and WKY rats (Muratani et al., 1991), suggesting no enhanced sensitivity of RVL vasomotor neurons to Ang II in SHRs. The bigger difference in response to Ang II between the two species is that microinjections of Ang II into CVL induce a greater decrease in AP in WKY rats than in SHRs. Further studies are required to define the role of Ang II's action on RVL-spinal vasomotor neurons in the generation and maintenance of hypertension.

*5. γ-Aminobutyric acid.* Altered operation of GABAergic inputs onto RVL vasomotor neurons may contribute to the generation and maintenance of many forms hypertension. GABA powerfully and rapidly inhibits RVL-spinal vasomotor neurons, and its action on these neurons represents the mechanism many sympathoinhibitory commands depend on for a rapid depressor response. Response of RVL vasomotor neurons to GABAergic inputs varies according to the functional states of the neurons and their environments. Thus, lower extracellular pH attenuates and higher pH enhances the GABA-induced inhibition (Sun and Reis, 1993a). The proton-induced attenuation of the neuronal response to GABA is associated with a depressed baroreflex inhibition of the vasomotor neurons (Sun and Reis, 1993a). Tissue acidosis, caused by hypercapnia and lactate accumulation, is known to occur during cerebral ischemia-hypoxia, especially of severe and/or long-term, and therefore may fine-tune and contribute to the ischemic-hypoxic sympathoexcitatory pressor response. Such pH changes may also be evoked by the use of several types of drugs. Functions of $GABA_A$ receptors also depend on protein tyrosine kinase-dependent phosphorylation (Stelzer et al., 1988; Moss et al., 1992; Valenzuela et al., 1995). Blocking protein kinase C of the vasomotor neurons, however, does not alter their response to GABA (Sun and Reis, 1994a). Another big influence is the redox effects on the $GABA_A$ receptors in some neurons (Lipton et al., 1996). However, their (*a*) potential effect on the GABAergic transmission onto RVL vasomotor neurons, (*b*) physiological and pathophysiological role, and (*c*) implications for therapy remain to be explored. Nevertheless, diminished GABAergic inputs onto RVL-spinal vasomotor neurons may underlie some forms of genetic hypertension. SHRs show smaller depressor response to microinjections of GABA into RVL than normotensive rats do (Kubo et al., 1986). In SHRs, inhibition of CVL, which contains GABAergic interneurons that project to RVL, has been found to have no effect on AP (Smith and Barron, 1990a). How-

ever, microinjections of Glut (Smith and Barron, 1990a; Muratani et al., 1993) or kainic acid (Yang et al., 1996) into CVL in SHRs have been reported to cause a greater depressor response (greater initial depressor response with kainic acid), suggesting that the GABAergic inputs from CVL are inactive in SHRs rather than damaged. Mean AP of SHRs and WKY rats decreases initially to a similar level of about 50 mmHg after the kainic acid administration into CVL (Yang et al., 1996), suggesting that activation of CVL neurons is still able to abolish the sympathetic tone in SHRs. In other studies, microinjections of bicuculline into RVL in SHRs increase AP dramatically (Muratani et al., 1993), suggesting that GABAergic inputs onto RVL vasomotor neurons are active in SHRs but may be insufficient. Restoring the GABAergic inputs onto RVL-spinal vasomotor neurons therefore represents the specific goal of pharmacological therapy in the treatment of hypertension. It has not been determined whether a gradual genetically timed loss of the functional GABAergic interneurons defines the development of the hypertension in SHRs. The possibility exists that environmental factors, such ischemic/hypoxic episodes and brain lesion, may gradually damage the GABAergic neurons or impair their function and thereby establish hypertension. Function of GABAergic system in other brain areas, such as in cortexes, has been shown to be transiently or permanently impaired by hypoxia, ischemia or focal lesion in several animal models and in humans (Romijin et al., 1988, 1992; Li et al., 1993; Verheul et al., 1993; Miehe et al., 1994). If this turns out to be the case, one strategy to prevent the development of hypertension and treat these forms of hypertension would be to avoid the hypoxic/ischemic incidents in vulnerable population and restore the GABAergic inputs.

The RVL-spinal vasomotor neurons represent one of the major targets for pharmacological treatments. Normalizing AP of the hypertensives is better achieved through correcting the wrong, whether it is enhanced sensitivity and inputs of Ang II, Glut and/or ACh, or a diminished synaptic transmission and sensitivity to GABA of RVL-spinal vasomotor neurons. The benefit of such therapeutics obviously depends on the importance of the altered mechanisms in hypertension and avoidance of disturbance of other vital functions (Sun and Reis, 1996d) during the treatment. Synergistic interaction of several putative neurotransmitters (many of which may be co-released) on the same vasomotor neurons (Agarwal and Calaresu, 1992) must be explored. Effects of frequent episodes of ischemia-hypoxia on the long-term regulation of AP must be evaluated. With more detailed understanding of the pathophysiology, we shall be in a better position to prevent the establishment of hypertension and to treat hypertensive patients.

### *B. Cardiac Failure and Shock*

Sympathetic activation in cardiac failure provides adrenergic drive to the failing myocardium and may facilitate arrhythmogenesis and progressive myocardial deterioration. Responses of RVL-spinal vasomotor neurons appear to be central to the majority of the supraspinal sympatho-responses. Manipulation of their activity through pharmacological means is indicated in the treatment of congestive heart failure, which is characterized by exaggeratedly increased SNA and suppressed baroreflexes (Manolis et al., 1995; Zucker et al., 1995; Wang et al., 1996). Elimination of the sympathetic hyperactivity can be achieved by the use of agents that inhibit RVL-spinal vasomotor neurons. RVL neurons may be targeted in the same way to reduce the syndrome and cerebrovascular risks associated with acute opioid withdrawal. Over-inhibition, however, may not be beneficial.

On the other hand, a manipulated increase in SNA and AP may be indicated under conditions such as endotoxic and hemorrhagic shock. Prevention of the cardiovascular collapse under such conditions can be achieved by a long-term infusion of vasoactive solution or better specifically by a combination of a direct excitation of RVL vasomotor neurons (e.g., TRH) and of a blockade of inhibitory inputs (e.g., opioids and adenosine) that inhibit RVL vasomotor neurons and thereby limit the expression of sympathetic drive. Opioid-like peptide system is activated by trauma and in endotoxic shock (Xu et al., 1992) and hemorrhagic shock. Inhibition of RVL vasomotor neurons under such conditions and opiate intoxication contribute greatly to decreases in sympathetic tone and cardiovascular collapse, and its blockade through pharmacological means is indicated in the treatment. For example, severe hemorrhage activates a portion of the PNMT-IR and PNMT-negative neurons in RVL, judging by the induced Fos immunoreactivity in these neurons (Dun et al., 1993). Acute loss of blood initially increases activity of RVL-spinal vasomotor neurons, SNA, and peripheral vascular resistance, until a abrupt vasodilation occurs when, for instance, more than 28% of blood volume is lost over a period of a few minutes in conscious rabbits (Ludbrook and Rutter, 1988). The abrupt decline in AP is associated with a sudden fall in SNA and marks the beginning of the fatal de-compensatory phase of severe hemorrhage. The relevance of the response to RVL vasomotor neurons is indicated by the evidence that endogenous opiate mechanisms are involved in this failure of sympathetic drive and that RVL vasomotor neurons are inhibited by the activated opiate inputs. It has been shown that central administration of an opiate receptor antagonist prevents the reversal of reflex vasoconstriction to vasodilatation in the de-compensatory phase of severe hemorrhage (Ludbrook and Rutter, 1988). The beneficial effect against severe hemorrhage can be achieved by bilateral

microinjections of naloxone (20 nmol/site in rabbits) into RVL (Morilak et al., 1990), although such changed activity and antagonism by naloxone during severe hemorrhage has not been monitored directly on singly identified RVL-spinal vasomotor neurons.

As mentioned in section IX. D., surviving rats have significantly more TRH in the medulla oblongata and other brain regions than do non-surviving animals after hemorrhagic shock (Mizobe and Okuda, 1988). Effects of TRH on preventing hemorrhagic shock depend, at least in part, on RVL vasomotor neurons, because i.c.v. TRH (10 $\mu$g) increases AP, HR and activities of the RVL neurons that show an increased firing rate to a fall in AP, whereas lesions of bilateral RVL areas abolish the i.c.v. TRH-induced cardiovascular responses in rats (Yan et al., 1992).

A direct involvement of RVL-spinal vasomotor neurons as the main target for a variety of cytokines to produce neurogenic cardiovascular responses including septic as well as hyperthermic shock (Störr et al., 1995; Kannan et al., 1996) remains to be investigated. The growing understanding of these fundamental mechanisms should prove very valuable in the clinical diagnosis and pharmacological treatments of a variety of cardiovascular diseases, including hypertension and cardiac failure.

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