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The physiological role of AT_1 receptors in the ventrolateral medulla

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Abstract

Neurons in the rostral and caudal parts of the ventrolateral medulla (VLM) play a pivotal role in the regulation of sympathetic vasomotor activity and blood pressure. Studies in several species, including humans, have shown that these regions contain a high density of AT_1 receptors specifically associated with neurons that regulate the sympathetic vasomotor outflow, or the secretion of vasopressin from the hypothalamus. It is well established that specific activation of AT_1 receptors by application of exogenous angiotensin II in the rostral and caudal VLM excites sympathoexcitatory and sympathoinhibitory neurons, respectively, but the physiological role of these receptors in the normal synaptic regulation of VLM neurons is not known. In this paper we review studies which have defined the effects of specific activation or blockade of these receptors on cardiovascular function, and discuss what these findings tell us with regard to the physiological role of AT_1 receptors in the VLM in the tonic and phasic regulation of sympathetic vasomotor activity and blood pressure.

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Introduction

The ventrolateral medulla (VLM) contains several different groups of neurons that play a major role in cardiovascular regulation. They consist of i) sympathoexcitatory neurons in the rostral VLM that project directly to the spinal sympathetic outflow, ii) sympathoinhibitory neurons in the caudal and intermediate parts of the VLM that project to and inhibit the rostral VLM sympathoexcitatory neurons, and iii) catecholamine-synthesising neurons of the A1 cells group that project to and excite vasopressin-synthesising neurons in the supraoptic and paraventricular nuclei in the hypothalamus. Although these three groups of neurons differ greatly with respect to their functional and anatomical properties, one common feature is that they all contain angiotensin type 1 (AT_1) receptors. Furthermore, as shown in Figure 1, AT_1 receptors in the medulla are specifically located in the VLM and in the nucleus tractus solitarius (NTS), another region that also plays a crucial role in cardiovascular regulation. In contrast, there is a very low density of AT_1 receptors in other medullary regions that subserve non-cardiovascular functions.

The striking association between AT_1 receptors and medullary cardiovascular nuclei raises the question as to the precise functions

of these receptors in cardiovascular regulation. This brief review will begin with a description of the distribution of AT_1 receptors in the VLM, but will then discuss mainly recent studies which have provided information on the functional effects of activation or blockade of these receptors, ranging from *in vivo* studies in whole animals to *in vitro* studies on single neurons. We shall then consider the fundamental question as to the normal physiological role of VLM AT_1 receptors in cardiovascular function. In this review we will not consider the role of these receptors in brain regions outside the VLM, which have been discussed in detail in other recent reviews (1,2).

Distribution of AT_1 receptors in the VLM

Studies using *in vitro* autoradiography have demonstrated a high density of angiotensin (Ang) receptor binding sites in the VLM of most species studied, including humans (3-5). These receptors are primarily of the AT_1 subtype (6,7). In the rat, *in vitro* autoradiography has revealed a relatively low density of AT_1 receptors in comparison to other species (8). Nevertheless, a recent study using an antibody against the AT_1 receptor has confirmed the existence of these receptors on neurons in the VLM of the rat and has shown that many of these neurons are also catecholamine neurons of the C1 group (9). There appear to be no published receptor binding or immunohistochemical studies showing the presence of AT_2 or Ang-(1-7) receptors in the VLM, although there is functional evidence in the rat for the existence of these receptor subtypes (10,11).

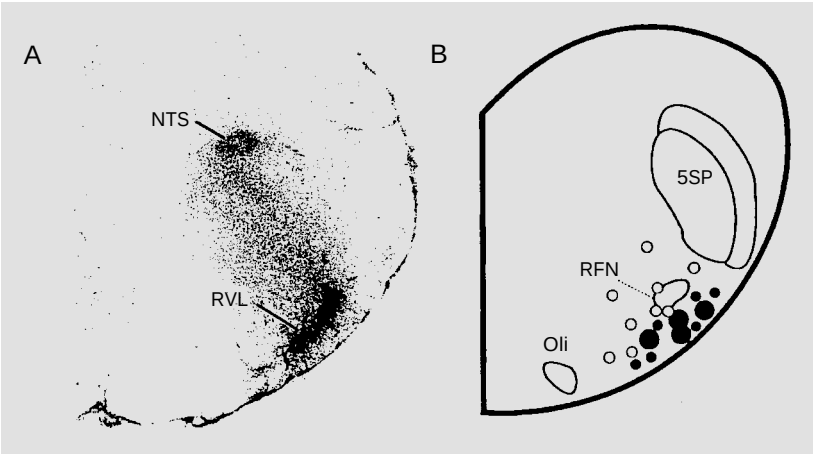

Figure 1 - *A*, Distribution of Ang II receptor binding sites in the rostral VLM (RVL) of the rabbit, using *in vitro* autoradiography (adapted from Ref. 3 and reprinted with the kind permission of the publisher). *B*, Distribution of sites in the rostral VLM of the rabbit at which microinjections of Ang II (20 pmol in 20 nl) elicited rises in mean arterial pressure of 6-20 mmHg (small filled circles) or more than 20 mmHg (large filled circles). The open circles indicate sites at which no significant change in mean arterial pressure was observed. (Adapted from Ref. 13, with the kind permission of the publisher). Note the close match between the receptor binding sites and the pressor sites. NTS, Nucleus tractus solitarius; RFN, retrofacial nucleus; 5SP, spinal trigeminal nucleus; Oli, inferior olivary nucleus.

Effects of application of exogenous Ang II to VLM neurons

In 1988, two studies were published that demonstrated for the first time that Ang II can directly excite pressor neurons in the VLM. Allen et al. (4) reported that microinjection of Ang II into the pressor region of the rostral VLM increased arterial pressure and heart rate, while Andreatta et al. (12) reported the same effect following application of Ang II to the nearby ventral surface. In a later and more detailed study by Sasaki and Dampney (13), it was found that the distribution of sites in the rostral VLM of the rabbit at which Ang II microinjection evoked increases in arterial pressure and renal sympathetic nerve activity closely matched the distribution of Ang II receptor binding sites (Figure 1), which, as discussed above, were later demonstrated to be AT_1 binding sites. Consistent with this, Hirooka et al. (14) found that the pressor and sympathoexcitatory response evoked by microinjection of Ang II into the rostral VLM was prevented by prior administration of the selective AT_1 receptor antagonist losartan into the same region (Figure 2).

Similarly, studies in the rat have confirmed that microinjection of Ang II into the

rostral VLM of this species also evoked a rise in arterial pressure (11,15). Furthermore, Chan and co-workers (16), using single-unit recording, found that many but not all rostral VLM neurons were excited by direct application of Ang II. These observations were confirmed by *in vitro* studies, which demonstrated using patch clamp recordings that Ang II evokes a depolarisation in some but not all spinally projecting neurons, an effect which is due to a reduction in potassium conductance (17). This effect was also blocked by administration of losartan, demonstrating that it is mediated by AT_1 receptors. A similar mechanism of action of Ang II on hypothalamic neurons has also been demonstrated (18).

With regard to the caudal and intermediate VLM, Sasaki and Dampney (13) found that microinjection of Ang II into this region evoked a fall in arterial pressure and renal sympathetic nerve activity. As with the rostral VLM, the distribution of sites evoking cardiovascular responses closely matched that of AT_1 receptor binding sites (6). The depressor and sympathoinhibitory response evoked by Ang II in this region can be explained as a consequence of activation of inhibitory interneurons that project directly to rostral VLM sympathoexcitatory neurons (19). Such interneurons have been identified in the caudal and intermediate VLM of the rabbit and rat (20-22). Anatomical studies have shown that these neurons are not catecholamine neurons, although they are co-localised with catecholamine neurons (22, 23).

Allen et al. (24) found that microinjection of Ang II into the caudal VLM also evoked a rise in the level of circulating vasopressin. This can be explained as a result of activation of caudal VLM neurons that project to and excite vasopressin-secreting neurons in the hypothalamic paraventricular and supraoptic nuclei. Unlike caudal VLM neurons that project to the rostral VLM, the hypothalamus-projecting neurons in the caudal VLM are mainly catecholamine neurons of the A1 group (25).

A recent study in the conscious rabbit used the method of immediate early gene expression to identify the population of neurons in the VLM that are activated by administration of Ang II into the fourth ventricle (26). These experiments were performed in both intact and sinoaortic denervated rabbits (to eliminate possible secondary effects on immediate early gene expression arising from blood pressure changes induced by the Ang II administration). In both groups of animals, Fos, the protein product of the immediate early gene c-*fos*, was detected in neurons in both the NTS and VLM, with a distribution that closely matched the distribution of AT_1 receptors in this region (Figure 3). The neurons in the VLM that were activated by Ang II consisted of both catecholamine and non-catecholamine neurons, as shown by double-labeling for tyrosine hydroxylase immunoreactivity (Figure 3). This study showed that approximately 60% of the rostral VLM neu-

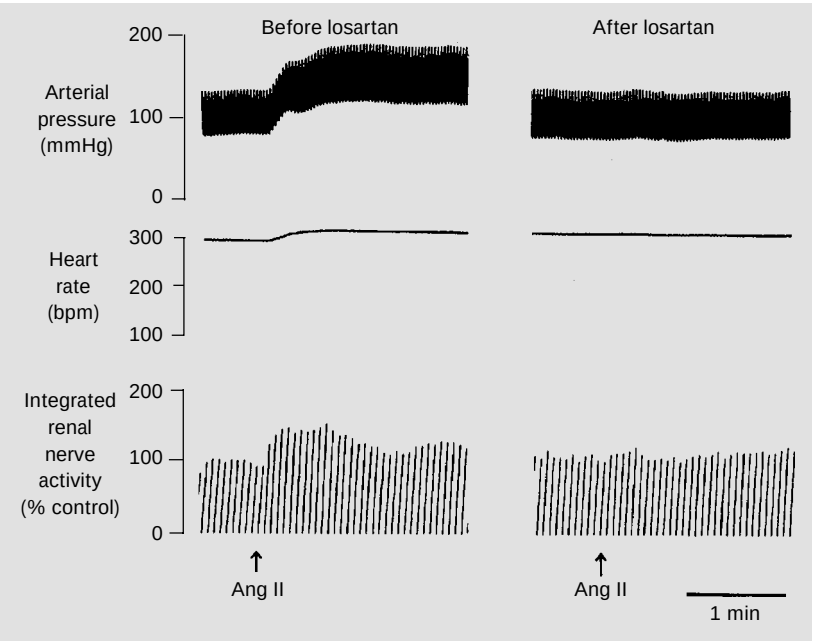

Figure 2 - Chart recording showing the effects on cardiovascular variables of microinjection of angiotensin II (Ang II, 40 pmol) into the pressor region in the rostral ventrolateral medulla before and after microinjection of losartan (1 nmol) into the same site (reprinted from Ref. 14, with the kind permission of the publisher).

rons that expressed Fos in response to Ang II were catecholamine neurons. It is interesting to note that a similar proportion (63%) of rostral VLM neurons that express Fos in response to baroreceptor unloading in the conscious rabbit are also catecholamine neurons (27). Thus, the results of these two studies using different experimental approaches indicate that although the majority of rostral VLM neurons with functional AT_1 receptors may be catecholamine neurons, this also appears to be the case for the general population of neurons within the rostral VLM that have a sympathoexcitatory function.

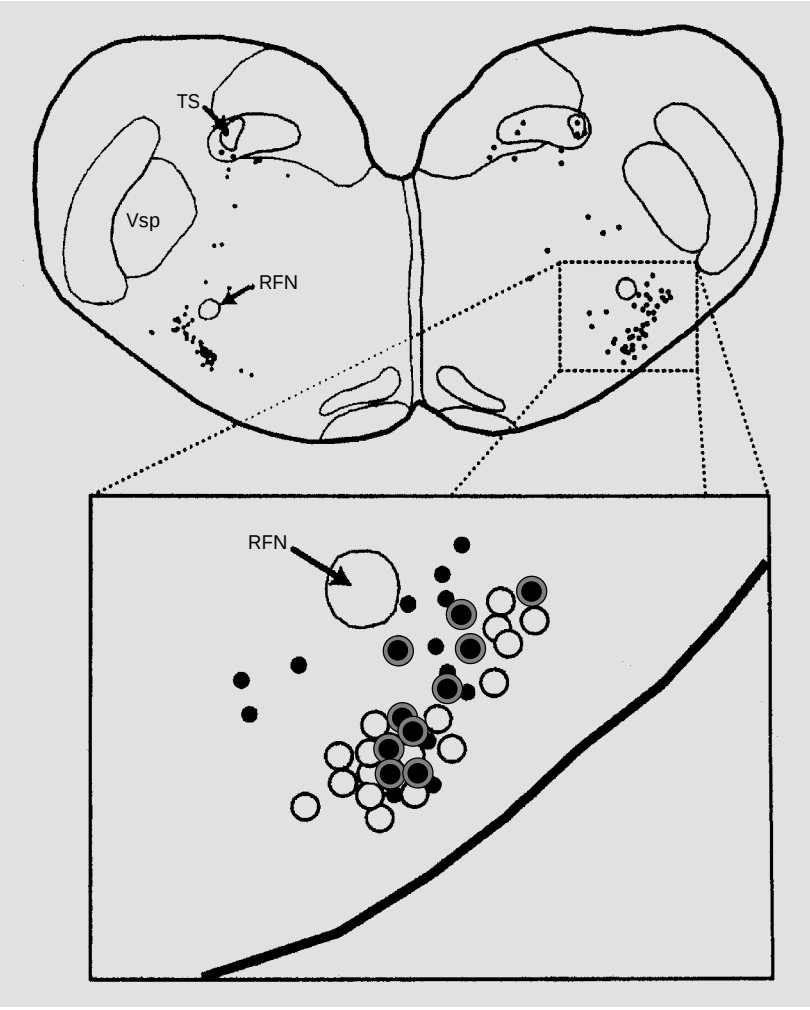

Figure 3 - *Upper panel*, Distribution of cells immunoreactive for Fos (indicated by small filled circles) in the medulla oblongata at the level of the rostral VLM following fourth ventricular infusion of Ang II in the conscious rabbit. *Lower panel*, Distribution of Fos-positive cells (small filled circles), cells immunoreactive for tyrosine hydroxylase (TH, a marker of catecholamine cells) (large open circles) and neurons immunoreactive for both Fos and TH (shaded circles enclosing small filled circles) in the rostral VLM in the region indicated by the rectangle in the upper panel. Vsp, Spinal nucleus of the trigeminal nucleus; RFN, retrofacial nucleus; TS, solitary tract. (Adapted from Ref. 26, with the kind permission of the publisher).

Similarly, neurons within the intermediate and caudal parts of the VLM that expressed Fos in response to Ang II also consist of catecholamine and non-catecholamine neurons (26). Thus, these findings are consistent with the results of the physiological studies described above indicating that both catecholamine and non-catecholamine neurons in the intermediate and caudal VLM can be activated by exogenous Ang II. The former group are likely to be the A1 neurons that project to vasopressin-secreting neurons in the supraoptic and paraventricular nuclei in the hypothalamus, while the latter are presumably inhibitory interneurons that project to rostral VLM sympathoexcitatory neurons.

An important question is the specificity of action of Ang II in the VLM. Although the anatomical and functional studies summarised above provide ample evidence that AT_1 receptors are associated with presympathetic vasomotor neurons in the rostral VLM, it is not clear whether they are also associated with neurons subserving other functions, such as respiratory regulation or antinociception, since such neurons are also located within this region (19). To test the possibility that AT_1 receptors in the rostral VLM may be associated with non-cardiovascular functions, Li et al. (28) determined the effects of microinjection of Ang II into the rostral VLM on phrenic nerve activity as well as arterial pressure. The results of these studies showed clearly that, although pressor responses evoked by microinjections of glutamate into the rostral VLM were invariably associated with changes in phrenic nerve activity, this was never the case with Ang II, even when large doses were injected. These results are consistent with the hypothesis that the pep-

tide acts exclusively on vasomotor neurons in this region.

Effects of blockade of AT_1 receptors in the VLM on resting sympathetic vasomotor activity

Sasaki and Dampney (13) first reported that unilateral microinjection of the peptidic Ang receptor antagonist [Sar[1],Thr[8]]Ang II resulted in a fall in arterial pressure and renal sympathetic nerve activity, suggesting that endogenous Ang II is tonically released in the rostral VLM and contributes to the resting activity of rostral VLM neurons and hence sympathetic activity. Later, Ito and Sved (29) showed that bilateral microinjections of [Sar[1],Thr[8]]Ang II or another peptidic antagonist, [Sar[1],Ile[8]]Ang II, resulted in a profound fall in arterial pressure, close to the level seen after complete blockade of the sympathetic vasomotor activity. On the basis of these observations, Ito and Sved (29) suggested that endogenous Ang II makes a very major contribution to the maintenance of sympathetic vasomotor tone. Recently, we have confirmed this observation and extended it by demonstrating that renal sympathetic nerve activity is also profoundly reduced (30). However, the sympathoinhibitory effects resulting from bilateral injections of [Sar[1],Thr[8]]Ang II or [Sar[1],Ile[8]]Ang II into the rostral VLM were not altered by prior blockade of glutamate or GABA receptors in this region (Figure 4), indicating that they are not mediated by presynaptic modulation of tonic glutamatergic or GABAergic activity. Thus, these findings suggest that [Sar[1],Thr[8]]Ang II and [Sar[1],Ile[8]]Ang II act postsynaptically to inhibit the tonic activity of rostral sympathoexcitatory neurons.

However, [Sar[1],Thr[8]]Ang II and [Sar[1], Ile[8]]Ang II are broad-spectrum Ang receptor antagonists, and therefore block AT_1, AT_2 and Ang-(1-7) receptors. In contrast to the effects of [Sar[1],Thr[8]]Ang II or [Sar[1],Ile[8]]Ang II, however, selective blockade of AT_1 or AT_2 receptors has little effect on resting arterial pressure and renal sympathetic nerve activity (11,14), at least under normal conditions. On the other hand, bilateral selective blockade of Ang-(1-7) receptors in the rostral VLM of the rat does result in a significant fall in arterial pressure (11), but the hypotensive effect is much less than that resulting from [Sar[1],Thr[8]]Ang II and

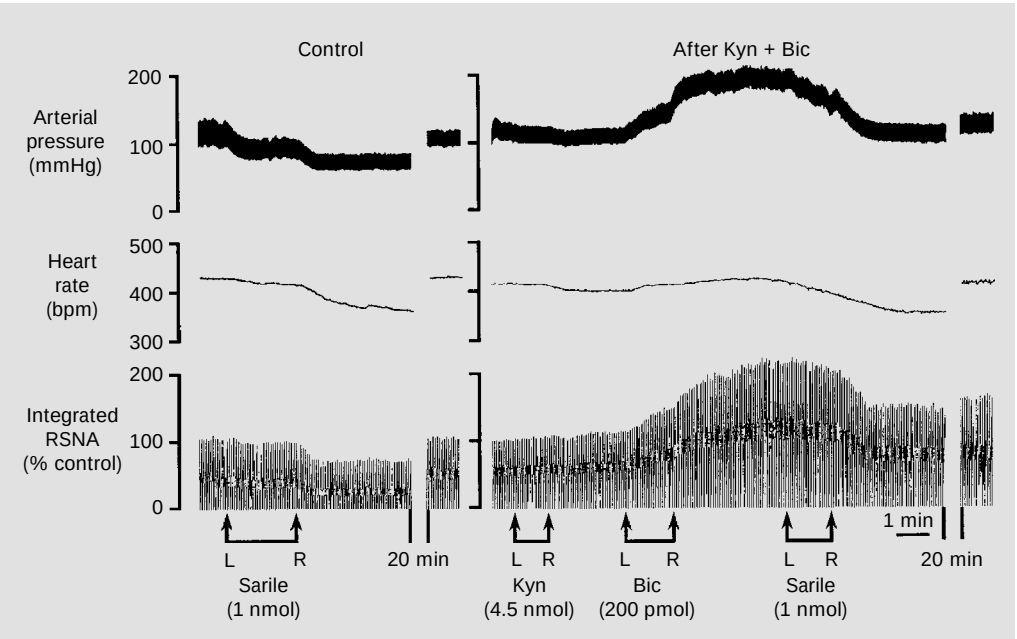

Figure 4 - Example of the effects on arterial pressure, heart rate and integrated renal sympathetic nerve activity (RSNA) evoked by bilateral microinjections of [Sar[1],Ile[8]]Ang II (Sarile) into the left (L) and right (R) rostral VLM, before and after bilateral injections of kynurenic acid (Kyn) plus bicuculline (Bic) into the same sites. Note that the depressor and sympathoinhibitory response evoked by [Sar[1], Ile[8]]Ang II was unaffected by prior injection of kynurenic acid plus bicuculline (reprinted from Ref. 30, with the kind permission of the publisher).

[Sar[1],Ile[8]]Ang II. Thus, it seems likely that the inhibitory effects of these compounds on rostral VLM sympathoexcitatory neurons are due to mechanisms that are independent of AT_1, AT_2 or Ang-(1-7) receptors. Further studies are required to elucidate these mechanisms.

There is much debate concerning the mechanisms that maintain the resting activity of rostral VLM sympathoexcitatory neu-

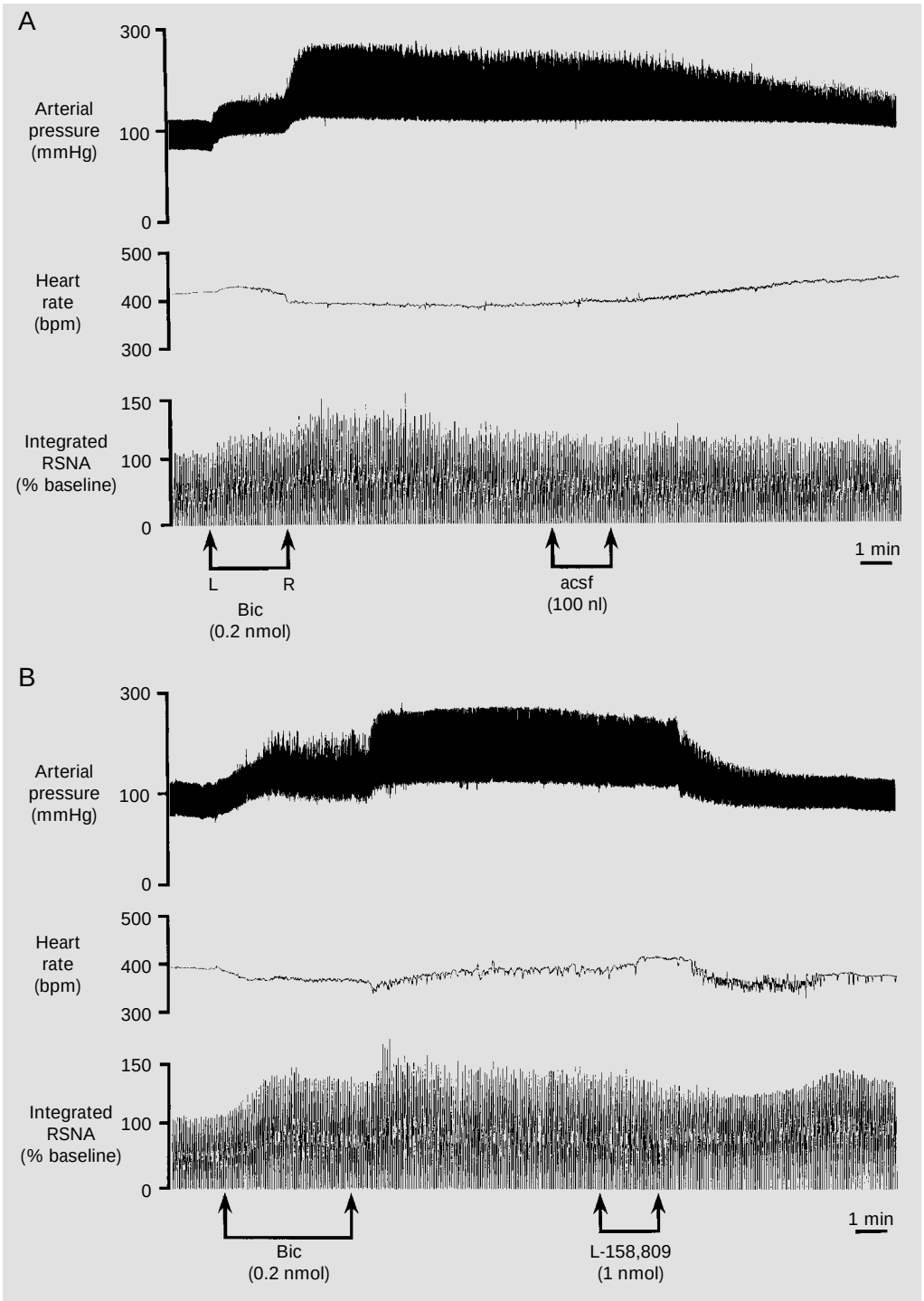

Figure 5 - Examples of the effects on arterial pressure, heart rate and integrated renal sympathetic nerve activity (RSNA) evoked by bilateral microinjections into the rostral VLM of either artificial cerebrospinal fluid (acsf, the vehicle solution) (A) or the AT_1 receptor antagonist L-158,809 (B) after prior bilateral microinjections of the GABA receptor antagonist bicuculline (Bic) into the same region. The paired arrows indicate the times of the first and second bilateral injections. Note that there was a decrease in arterial pressure, heart rate and RSNA following injections of L-158,809, indicating that there is a tonic excitatory action on rostral VLM sympathoexcitatory neurons mediated by AT_1 receptors under conditions when GABAergic inhibitory inputs are blocked.

rons (19,31,32). It is clear, however, that these neurons are subject to a tonic GABAergic inhibition (33). In the presence of GABA receptor blockade, a powerful tonic excitatory effect on rostral VLM neurons is unmasked, resulting in a large increase of sympathetic activity and arterial pressure (30,34). Recently, we have obtained evidence that AT_1 receptors do make a small contribution to the maintenance of the resting activity of rostral VLM sympathoexcitatory neurons, which can be revealed when the effect of tonic GABAergic inhibition of these neurons is removed. As shown in Figure 5A, bilateral microinjections of bicuculline into the rostral VLM pressor region resulted in a large and sustained rise in sympathetic activity and arterial pressure. However, when a selective AT_1 receptor antagonist (losartan or L-158,809, gift of Merck Co., Rahway, NJ, USA) was injected during the period when the arterial pressure and sympathetic activity were elevated following GABA receptor blockade in the rostral VLM, both arterial pressure and renal sympathetic nerve activity decreased substantially (Figures 5B, 6). Thus, tonic activation of AT_1 receptors may contribute to the resting activity of rostral VLM neurons, but this effect is only apparent under conditions where the tonic inhibition of these neurons is eliminated.

Effects of blockade of AT_1 receptors in the VLM on synaptic regulation of rostral VLM sympathoexcitatory neurons

Although there is abundant evidence, as summarised above, that activation of AT_1 receptors excites cardiovascular neurons in the VLM, the major question that remains is: what is the normal physiological role of these receptors in the synaptic regulation of these neurons? Synaptic excitation of rostral VLM neurons that occurs in response to stimulation of a wide variety of peripheral receptors as well as some supramedullary regions appears to be mediated primarily by glutamate receptors, since it is blocked by kynurenic acid, a broad-spectrum ionotropic glutamate receptor antagonist (19,31,35-38). At the same time, there is evidence that some excitatory inputs to rostral VLM sympathoexcitatory neurons are mediated by non-glutamate receptors. In particular, Kiely and Gordon (39) found that the pressor response evoked by stimulation of certain hypothalamic areas (the paraventricular nucleus (PVN) and the perifornical area) is reduced by non-specific blockade of synaptic transmission in the rostral VLM, but not by blockade of glutamate receptors in this region.

These observations therefore raise the possibility that excitatory inputs to the rostral VLM sympathoexcitatory neurons are mediated by AT_1 receptors. To test this hypothesis, we have recently determined the

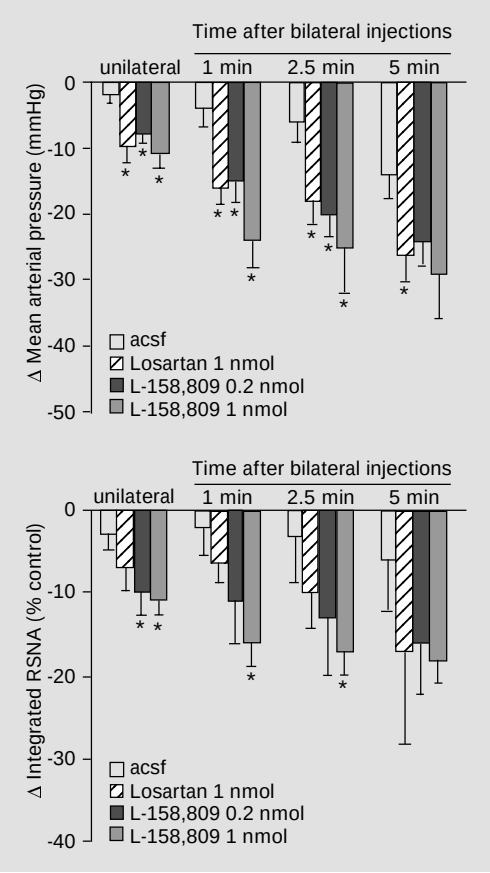

Figure 6 - Histogram summarising the effects of unilateral and bilateral injections into the rostral VLM of either artificial cerebrospinal fluid (acsf, N = 6) or the AT_1 receptor antagonists losartan (1 nmol, N = 6) or L-158,809 (0.2 nmol, N = 6 or 1.0 nmol, N = 7) on arterial pressure and renal sympathetic nerve activity (RSNA). In all cases the GABA receptor antagonist bicuculline had been injected bilaterally into the rostral VLM approximately 10 min before the injections of these different compounds. *P<0.05 for the effects of losartan or L-158,809 compared with that of the vehicle solution (acsf).

effect of selective blockade of AT_1 receptors in the rostral VLM on the cardiovascular response evoked by activation of the hypothalamic PVN (40). For this purpose, microinjections of bicuculline were made into the PVN, since this has been shown to be a highly effective and reproducible means of activating PVN neurons (41,42).

This study demonstrated that the increase in arterial pressure, heart rate and renal sympathetic nerve activity that was evoked by a unilateral microinjection of bicuculline into the PVN was reduced by approximately 50% after microinjection of the potent neuroinhibitory compound muscimol into the ipsilateral rostral VLM. However, the response was unaffected by muscimol injection into the contralateral PVN, which is consistent with anatomical studies showing that the descending pathway from the PVN to the rostral VLM is almost entirely ipsilateral (43). Thus, these experiments demonstrated that the pressor and sympathoexcitatory response evoked from the PVN is partly mediated by the ipsilateral rostral VLM, and partly via a descending pathway that is independent of the rostral VLM. The most interesting observation, however, was that the response evoked from the PVN was attenuated by about 50% following microinjection of the selective AT_1 receptor antagonists losartan or L-158,809 into the ipsilateral rostral VLM, but was unaffected by microinjection of kynurenic acid into the same region. In contrast to the response evoked from the hypothalamus, the sympathoexcitatory response evoked reflexly by stimulation of the sciatic nerve (the somatosympathoexcitatory reflex) was unaffected by losartan or L-158,809 in the rostral VLM, but was abolished by kynurenic acid in this region. Thus, these findings suggest that the excitatory input to rostral VLM sympathoexcitatory neurons arising from activation of the hypothalamic PVN, but not that reflexly evoked by stimulation of sciatic nerve afferents, is mediated by AT_1 receptors.

Many questions remain unanswered, however, as to the role of AT_1 receptors in the rostral VLM. For example, assuming that Ang II is the endogenous ligand for these receptors, what is the source of Ang II that is released in response to activation of the PVN? There is evidence that both the PVN and the lateral parabrachial nucleus contain neurons that are immunoreactive for Ang II (44). Both of these nuclei contain neurons that project to the rostral VLM (19), but it has not been shown whether some of these neurons are also Ang II-containing neurons. Alternatively, Lippoldt et al. (45) have proposed that Ang II can be formed in the extracellular fluid from angiotensinogen, which itself is produced in astrocytes. Thus, it is possible that Ang II in the rostral VLM is not formed within the neurons, but is instead taken up from the extracellular fluid into nerve terminals from which it is subsequently released.

Another important question is whether Ang II is the sole neurotransmitter released from nerve terminals in response to PVN activation, or else is a co-transmitter together with another neurotransmitter such as glutamate. This possibility is consistent with the fact that Ang II and glutamate appear to be co-transmitters in the angiotensinergic pathway from the subfornical organ to the PVN (2).

A further question is whether AT_1 receptors in the rostral VLM mediate synaptic excitatory inputs only from the PVN, or whether they have a more generalised role in synaptic transmission. Given that excitatory inputs to the rostral VLM sympathoexcitatory neurons that originate from peripheral receptors and/or are relayed via the NTS are, at least in every case so far examined, dependent upon glutamate receptors in the rostral VLM (e.g., Refs. 35-38 and 40), it is possible that the AT_1 receptors have a much more specific function, perhaps mediating inputs only from certain specific supramedullary regions, including the PVN. Even if that is the case, however, this does not in-

clude all supramedullary inputs, because it is established that some excitatory inputs to rostral VLM sympathoexcitatory neurons arising from or passing through the hypothalamus are also glutamatergic (35).

A final possibility that should be briefly considered is whether AT_1 receptors in the rostral VLM play a functionally important role only under conditions of stress. This would be consistent with the finding that they mediate sympathoexcitatory responses originating from the PVN, since it is well known that this nucleus is activated during stress. Further, in a recent study in the conscious rabbit it was found that administration of losartan into the fourth ventricle resulted in an increase in renal sympathetic nerve activity under conditions of baroreceptor unloading and hypoxia, but not under resting conditions (46,47). Although the site of action of losartan in these studies is unknown (it could be the NTS, caudal or rostral VLM or even A5 area in the pons), the results are at least consistent with the hypothesis that medullary AT_1 receptors are functionally important only under conditions of stress, such as hypotension or hypoxia. At present, however, this hypothesis is speculative.

In conclusion, the results of studies to date have demonstrated that AT_1 receptors in the rostral VLM play a significant role in the regulation of sympathetic vasomotor activity or arterial pressure, at least under some physiological conditions. Further studies will be needed, however, to elucidate their precise physiological role.

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