Inhibition of neurons in commissural nucleus of solitary tract reduces sympathetic nerve activity in SHR

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Sato, Monica A., Eduardo Colombari, and Shaun F. Morrison. Inhibition of neurons in commissural nucleus of the solitary tract reduces sympathetic nerve activity in SHR. Am J Physiol Heart Circ Physiol 282: H1679–H1684, 2002; 10.1152/ajpheart.00619.2001.—Neurons in the commissural nucleus of the solitary tract (commNTS) play an important role in certain cardiovascular responses dependent on sympathetic vasoconstrictor activation, including the arterial chemoreceptor reflex. Electrolytic lesions of the commNTS elicit a fall in arterial pressure (AP) in spontaneously hypertensive rats (SHR). To determine whether the latter result 1) arose from elimination of commNTS neuronal activity rather than en passant axons and 2) was accompanied by a reduction in sympathetic nerve activity, we evaluated the effect of inhibition of neurons in the commNTS on basal splanchnic sympathetic nerve activity (SNA), AP, and heart rate (HR) in SHR, Wistar-Kyoto (WKY), and Sprague-Dawley (SD) rats. In chloralose-anesthetized, paralyzed, and artificially ventilated SHR, microinjection of GABA into the commNTS markedly decreased splanchnic SNA, AP, and HR. The reductions in SNA and AP following similar microinjections in WKY and SD rats were significantly less than those in SHR. Our findings suggest that tonically active neurons in the commNTS contribute to the maintenance of SNA and the hypertension in SHR. The level of tonic discharge of these commNTS neurons in normotensive WKY and SD rats may be lower than in SHR.

THE NUCLEUS OF THE SOLITARY TRACT (NTS) is the initial synaptic integration site for baroreceptor and arterial chemoreceptor afferent inputs (5, 9, 39). Baroreceptor afferent activation elicits depressor responses via a sympathoinhibitory pathway from the dorsomedial NTS that involves activation of GABAergic neurons (1, 17, 18, 20, 40) in the caudal ventrolateral medulla (CVLM) (2, 12, 22) that inhibit vasoconstrictor sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM). Stimulation of arterial chemoreceptors evokes pressor responses via a sympathoexcitatory pathway from the commissural NTS (commNTS) that excites vasoconstrictor sympathetic premotor neurons in the RVLM either directly (21) or through brainstem pathways that involve neurons in the A5 region (13). Although their relationship to the chemoreceptor reflex is not known, the presence of sympathoexcitatory neurons in commNTS has been suggested by: 1) the increase in arterial pressure (AP) elicited by microinjection of glutamate into commNTS (8, 26, 31) and 2) the ability of inhibition of commNTS neurons to block both afferent stimulation-evoked increases in AP (24) and those evoked by inhibition of neurons in the CVLM (30).

Although electrolytic lesions in commNTS abolish the pressor response evoked by stimulation of the arterial chemoreceptor reflex with potassium cyanide in both normotensive rats and spontaneously hypertensive rats (SHR) (6, 37), it was only in SHR that such lesions reduced the level of basal AP. These data raise the possibility that a tonically elevated level of discharge of commNTS neurons in SHR contributes to the maintenance of hypertension in this model. In the present study, we sought to provide further support for this hypothesis, by determining 1) whether the depressor effects of commNTS lesions are due specifically to inactivation of neurons rather than en passant fibers in commNTS and 2) whether a reduction in vasoconstrictor sympathetic nerve activity (SNA) accompanies the fall in AP produced by interruption of activity in commNTS neurons. A preliminary report of these results has appeared (36).

METHODS

Experiments were performed on adult male SHR (14–16 wk old, 300–350 g) and Wistar-Kyoto (WKY) rats (14–16 wk old, 350–400 g) obtained from Taconic Farms and on Sprague-Dawley (SD) rats (350–400 g) supplied by Charles River. The latter group of animals was selected to include a normotensive strain that was genetically unrelated to the SHR. We reasoned that differences directly related to the hypertension in the SHR should also be demonstrable between SHR and normotensive strains other than the WKY rat. Animals were initially anesthetized with 3% isoflurane in 100% O2, the femoral artery and vein were cannulated for AP measurement and drug administration, respectively, and anesthesia was maintained with chloralose (60 mg/kg iv)

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RESULTS

Responses to microinjection of GABA into the commNTS. Figure 1 illustrates the effects of GABA microinjection into the commNTS in a SHR and a WKY rat. Within 10 s after the microinjection of GABA (1.8 nmol in 36 nl) into the commNTS in the SHR, splanchnic SNA began to decline and this was followed by decreases in AP and HR (Fig. 1A). Cardiovascular parameters returned to control levels within 2–3 min, likely due in part to local uptake or metabolism of GABA (38). In contrast, a similar microinjection of GABA in the WKY rat produced only a small and delayed reduction in SNA, no change in AP, and a modest bradycardia (Fig. 1B). In all six SHR, inhibition of neurons in the commNTS with microinjections of GABA reduced the splanchnic SNA (P < 0.001; Figs. 1 and 2), MAP (P < 0.001), and HR (P < 0.001). In six WKY rats, microinjection of GABA into commNTS produced a fall in HR (Figs. 1 and 2), but no change in SNA or MAP. Similar microinjections in four SD rats had no effect on splanchnic SNA, MAP, or HR.

Control MAP was significantly (P < 0.01) higher in the SHR (163 ± 10 mmHg) than in WKY (109 ± 6 mmHg) or SD (115 ± 4 mmHg) rats. Basal levels of splanchnic SNA were also greater (P < 0.05) in SHR than in either WKY or SD rats. There was no difference in basal HR among the three groups of rats (SHR: 415 ± 11 beats/min; WKY: 430 ± 23 beats/min; SD: 406 ± 45 beats/min). The reduction in splanchnic SNA produced by inhibition of neurons in the commNTS in the SHR (−31 ± 7% of control) was significantly (P < 0.01) greater than that in WKY (−8 ± 2% control) or SD (−3 ± 2% control) rats (Fig. 2A). This decrease in splanchnic SNA in the SHR was accompanied by a significantly (P < 0.05) greater fall in MAP (−48 ± 12 mmHg) compared with those elicited by microinjection of GABA into the commNTS of WKY (−11 ± 4 mmHg) or SD (−3 ± 5 mmHg) rats (Fig. 2B). The minimum MAPs following the microinjections of GABA into the commNTS were not different between the SHR and the normotensive groups (SHR: 115 ± 14 mmHg; WKY: 98 ± 5 mmHg; SD: 118 ± 6 mmHg). Microinjection of GABA into commNTS significantly (P < 0.05) decreased the HR in SHR (−55 ± 14 to 360 ± 20 beats/min; Fig. 2C) and WKY rats (−29 ± 12 to 401 ± 23 beats/min), but not in SD rats (−9 ± 10 to 397 ± 51 beats/min). The mean minimum HRs during inhibition of commNTS neurons were not different among strains.

Localization of GABA microinjection sites. The sites of fast green dye deposits marking the GABA microinjection sites in the three strains of animals are illustrated in Fig. 3. In all cases, the fast green dye deposits were located in the commNTS (34). This site corresponds to that in which electrolytic lesions reduced MAP in conscious SHR (37).

DISCUSSION

The major finding of this study is that inhibition of neurons in the commNTS reduced splanchnic SNA and...
MAP in SHR to a significantly greater degree than in normotensive WKY or SD rats. These results comprise the first evidence for a potential involvement of neurons in commNTS in the maintenance of elevated blood pressure and SNA in SHR.

In this study, we observed that inhibition of commNTS neurons had no effect on the splanchnic SNA or MAP in WKY and SD rats. The similarity between these results and those in our previous studies, in which electrolytic lesions of the commNTS produced no change in MAP in conscious, normotensive SD, or Wistar rats (6, 37) suggests that anesthesia is unlikely to account for the absence of an effect of GABA microinjection in the commNTS of normotensive animals in the present study. Similarly, excitation of commNTS neurons has been shown to increase MAP in both unanesthetized and urethane-anesthetized rats (8). As with all such microinjection studies, we cannot completely discount the possibility that GABA may have influenced neurons in the vicinity of commNTS to

Fig. 1. Responses of a spontaneously hypertensive rat (SHR) and a Wistar-Kyoto (WKY) rat to microinjection of GABA into the commissural nucleus of the solitary tract (NTS) (GABA commNTS). A: oscillographic traces recorded from an SHR. Top, splanchnic sympathetic nerve activity (SPL SNA); bottom, integrated splanchnic nerve activity (int SPL SNA, %control), arterial pressure (AP, mmHg), and heart rate (HR, beats/min) during microinjection of GABA (arrow) into commNTS. B: same traces as in A, recorded from a WKY rat. Vertical calibration is 5 μV for the SPL SNA traces, horizontal calibration is 60 s.

Fig. 2. Changes in cardiovascular parameters elicited by inhibition of neurons in commNTS. A: mean percent changes from control splanchnic sympathetic nerve activity (Δ%SNA) in SHR (open bar), WKY (solid bar), and SD (shaded bar) during maximum effect of GABA microinjection into commNTS. B: mean changes in mean arterial pressure (ΔMAP) under same conditions as in A. C: mean changes in HR (ΔHR) under same conditions as in A. Histogram values are means ± SE. *Significantly (P < 0.01) different from WKY and SD rats; **significantly (P < 0.05) different from WKY and SD rats.
produce the effects observed in this study. From a previous study (11), we expect that an effective concentration of GABA in the commNTS neurons in the normotensive strains, the absence of a fall in MAP in the normotensive strains after electrolytic lesions of commNTS (6, 37) would make this possibility seem less likely. Whether the commNTS neurons contributing to the support of elevated SNA and MAP in SHR are those that mediate the sympathetic activation during peripheral chemoreceptor activation (4, 13, 21, 35) remains to be determined. It is of interest, however, that altered synchronization between respiratory and sympathetic outflows during chemoreceptor reflexes have been described in the SHR (7, 10), although this alteration does not appear to be expressed as a difference in the amplitudes of the chemoreceptor reflex-evoked pressor responses between SHR and WKY rats (14, 37).

Ito et al. (15) have demonstrated an exaggerated, kynurenic acid-sensitive excitatory input to sympathetic, vasomotor neurons in the RVLM of SHR, although the source of this input has not been identified. Similar to our microinjections of GABA into commNTS in the current study, microinjection of kynurenic acid into the RVLM of normotensive rats had little effect on MAP. These results led Ito et al. (15) to propose that an imbalance of inhibitory and glutamate-mediated, excitatory influences on RVLM vasomotor neurons contributes to the elevated MAP in SHR. Neuroanatomical (32, 33, 42) and electrophysiological (21) evidence supports a direct projection from the commNTS to the RVLM. The commNTS is the primary termination site of peripheral chemoreceptor afferents (5, 9), and commNTS neurons are necessary for the sympathoexcitation and pressor responses evoked by peripheral hypoxia (13, 35). The latter responses, in turn, are mediated by activation of excitatory amino acid receptors in the RVLM (3, 22, 27). An increased sensitivity of arterial chemoreceptors to hypoxia in SHR (10), an altered sympathetic response to hypoxia in SHR (7), and enhanced depressor responses to hyperoxia in hypertensive subjects (16, 41) have suggested an involvement of the arterial chemoreceptor reflex in hypertension. In contrast, activation of peripheral chemoreceptors elicits similar pressor responses in SHR and WKY rats (14, 37). Taken together, these findings are consistent with a model in which neurons in the commNTS contribute, through a glutamate-mediated input, to an enhanced excitation of RVLM vasomotor neurons in SHR. The potential interaction of the peripheral chemoreceptor reflex with such NTS sympathoexcitatory neurons remains to be determined.

In the present study, HR also decreased after microinjection of GABA into the commNTS in SHR and WKY, but not in SD rats. If this result is indicative of a reduction in cardiac SNA, it would suggest that the commNTS neurons in the SHR and WKY play a role in maintaining cardiac sympathetic outflow as well as the elevated level of vasoconstrictor SNA seen in this and previous comparisons of SHR and WKY (19, 25, 28, 29). Although the greater depressor response in the SHR could be accounted for by a markedly reduced sensitivity to the inhibitory effects of GABA in the commNTS neurons in the normotensive strains, after electrolytic lesions of commNTS (6, 37) would make this possibility seem less likely. Whether the commNTS neurons contributing to the support of elevated SNA and MAP in SHR are those that mediate the sympathetic activation during peripheral chemoreceptor activation (4, 13, 21, 35) remains to be determined.
that to vasoconstrictor targets (i.e., that represented by the splanchnic SNA).

In summary, our present data show that tonically active neurons in commNTS contribute to the maintenance of elevated SNA and high blood pressure in SHR and that if such neurons are present in normotensive WKY and SD strains, their level of tonic discharge is likely to be markedly lower than in SHR. These results implicate a population of sympathoexcitatory neurons in commNTS in hypertension in the SHR.

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