Reduced GABA inhibition of sympathetic function in renal-wrapped hypertensive rats

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Martin, Douglas S., and Joseph R. Haywood. Reduced GABA inhibition of sympathetic function in renal-wrapped hypertensive rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1523–R1529, 1998.—Animals with bilateral cannulas in the paraventricular nucleus were made hypertensive by a one-kidney, figure eight renal wrap procedure or sham operated. Femoral artery and vein catheters were inserted for arterial pressure measurement and plasma catecholamine determination. After recovery and 4 days after hypertension surgery, bicuculline methiodide or muscimol was microinjected into the paraventricular nucleus. In some rats, nitroprusside was infused intravenously to reflexly stimulate the sympathetic nervous system. In control rats, bicuculline increased blood pressure, heart rate, and plasma norepinephrine and epinephrine concentrations. In contrast, in hypertensive rats blood pressure did not change while the heart rate response was maintained. Plasma norepinephrine and epinephrine responses were reduced 75 and 68 %, respectively. Muscimol injections decreased arterial pressure in the hypertensive rats. Heart rate responses to nitroprusside were similar in the two groups of rats, while the plasma catecholamine responses were attenuated in the hypertensive animals. These data suggest that GABA function in the paraventricular nucleus is reduced in renal wrap hypertension.

bicuculline; plasma catecholamines; paraventricular nucleus

SODIUM-DEPENDENT MODELS of hypertension, such as the one-kidney, figure eight renal wrap, are associated with an increased sympathetic nervous system function (13, 25, 37). Several lines of evidence have suggested that the paraventricular nucleus of the hypothalamus (PVN) participates in the central nervous system pathways involved in sodium-induced hypertension. Stimulation of the PVN leads to an increase in arterial pressure that is mediated by the sympathetic nervous system (16, 19). In addition, anatomic connections have been demonstrated between salt-sensitive areas in the forebrain (i.e., the organum vasculosum of the lamina terminalis, the median preoptic nucleus, and the subfornical organ) and the PVN (15). Finally, lesions of the PVN reverse or attenuate the development of the DOCA-salt model of hypertension (10, 23). Recently, ablation of the PVN has been shown to lower blood pressure in figure eight renal-wrapped hypertensive rats.

METHODOLOGY

Animal maintenance and surgery. Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 275–300 g were used in this study. Animals were maintained on a 14:10-h light-dark cycle in temperature-controlled quarters. Rats were maintained on an ad libitum water intake and normal sodium diet (Teklad, 100 μeq/g chow). All studies were performed according to the American Physiological Society's Guiding Principles for the Use of Animals in Research and Teaching and the Federation of American Societies for Experimental Biology Statement of Principles on the Use of Animals in Research and Teaching. The University of Texas Health Sciences Center at San Antonio is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

Rats were anesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg) and were placed in a Kopf stereotoxic instrument (David Kopf Instruments, Tujunga, CA) for instrumentation with a guide cannula directed bilaterally toward the PVN as described previously (19, 20). Five days later, the animals were anesthetized with methoxyflurane and subjected to a figure eight Grollman renal wrap procedure on the left kidney and a contralateral nephrectomy (11). Control animals underwent a sham operation, which
consisted of a unilateral nephrectomy. Two days after renal surgery, the animals were prepared with femoral arterial and venous catheters as described previously (19, 20). A total of 13 sham-operated and 20 renal-wrapped animals were used in this study.

Experimental protocol. The rats were housed in their recording cages for 24 h before the experiment. Four days after renal surgery, the arterial catheter was connected to a pressure transducer (Cobe Laboratories, Lakewood, CO) for the measurement of arterial blood pressure. Heart rate was derived from the pulsatile blood pressure signal by means of a cardiotachometer (Sensormedics, Anaheim, CA). Baseline values were recorded for at least 60 min before any interventions.

The microinjection apparatus consisted of 15-mm stainless steel injectors connected to 1.0-µl microsyringes (Hamilton, Reno, NV) by PE-20 tubing. The injectors were back filled with solutions of bicuculline methiodide (BMI) (1 mM) or muscimol (2 mM). The injectors were inserted into the guide cannulas, and sufficient time was allowed for blood pressure and heart rate to return to control levels. Fifty nanoliters were then injected manually over 1 min for a total dose of 50 pmol of BMI or 100 pmol of muscimol per site. In some rats, after the blood pressure and heart rate of the animal had recovered from the microinjection, an intravenous infusion of nitroprusside (100 µg/ml) was begun. The rate of infusion was titrated to produce a decrease in arterial pressure of 30–35 mmHg in each animal. One-milliliter arterial blood samples were drawn during the baseline period and during the plateau phase of the responses to BMI or nitroprusside (~3–5 min). The plasma was collected for the radioenzymatic assay of plasma norepinephrine (NE) and epinephrine (Epi) (24). The red blood cells from each sample were resuspended in 0.9% saline and given back to the animal before the next blood sample.

Data analysis. At the conclusion of the experiments, the animals were deeply anesthetized with pentobarbital sodium (Abbott Laboratories, Chicago, IL). The rats were then perfused transcardially with 0.9% saline followed by 10% buffered Formalin solution. Six-micrometer frozen sections were cut through the region of the PVN with a freezing microtome. The sections were stained with cresyl violet for identification of the injector placement. The injection sites are illustrated in the schematic in Fig. 1 according to the description of the hypothalamus by Swanson (36).

Data are presented as means ± SE. Statistical analysis was performed using Statview (Abacus Concepts, Berkeley, CA). Changes in variables among groups were analyzed with two-factor analysis of variance. Repeated-measures analysis of variance was used to determine the effect of drug administration between normotensive and hypertensive rats.

RESULTS

Responses to BMI. In the normotensive animals (n = 8), resting mean arterial pressure and heart rate were 117 ± 2 mmHg and 391 ± 9 beats/min. Resting blood pressure was significantly elevated in the rats (n = 10) subjected to the renal wrap procedure (145 ± 2 mmHg), whereas resting heart rate (368 ± 14 beats/min) was not significantly different from control animals. Microinjection of BMI into the PVN significantly increased arterial pressure to 136 ± 3 mmHg and heart rate to 464 ± 16 beats/min (Fig. 2) in the normotensive rats. This rise in arterial pressure and heart rate was rapid, occurring within 30–60 s. The pressor response reached a peak between 2 and 3 min. In contrast, in renal-wrapped animals, PVN stimulation with BMI increased arterial blood pressure to 148 ± 2 mmHg. The BMI microinjections in the hypertensive animals caused a significant increase in heart rate to 432 ± 12 beats/min, which was not different from that of the control group.

Fig. 1. Schematic representations of coronal sections of rat brain illustrate injection sites for sham-operated (●) and renal-wrapped animals (■) in the region of the paraventricular nucleus (PVN). Sections shown range from rostral (−1.33 mm from bregma) to caudal (−2.00 from bregma) regions of the PVN according to the atlas of Swanson (36). AHA, anterior hypothalamic area; f, fornix; VMH, ventromedial hypothalamus; SON, supraoptic nucleus.
The time course of the rise in heart rate was not different in normotensive and hypertensive animals. Thus, in the renal-wrapped hypertensive animals, the blood pressure responses to BMI disinhibition of the PVN were blunted while the heart rate responses remained intact. In both normotensive and hypertensive animals there was a stereotypical behavior consisting of facial grooming during the sustained increase in blood pressure and heart rate.

Basal levels of NE were 264 ± 18 pg/ml in the sham-operated rats and 180 ± 27 pg/ml in the hypertensive animals; the values were significantly different. Plasma Epi concentrations were similar in the normotensive (183 ± 27 pg/ml) and the hypertensive rats (159 ± 26 pg/ml). Administration of BMI in normotensive rats resulted in increases in plasma NE and Epi to 496 ± 77 pg/ml and 794 ± 212 pg/ml (Fig. 3). In contrast, in the hypertensive rats BMI induced an increase in NE to 239 ± 21 pg/ml and Epi to 353 ± 47 pg/ml that was significantly attenuated. Thus the BMI-induced elevations in plasma NE and Epi were reduced by 75 and 68%, respectively, in rats subjected to figure eight renal wrap hypertension.

Dispersal and muscimol. Muscimol caused no change in arterial pressure in the normotensive (n = 5) rats (Table 1). In the hypertensive animals (n = 8), there was an initial reduction in blood pressure of 5 ± 2 mmHg within 4 min that further increased to 9 ± 3 mmHg over the next 12 min. Neither group of rats experienced a significant reduction in heart rate.

DISCUSSION

Although a number of neurotransmitter systems may be involved in the pathogenesis of hypertension, alterations in central GABAergic function may play an integral role in this process. GABA stimulants administered intracerebroventricularly, such as muscimol or diazepam, as well as inhibitors of GABA metabolism lower arterial pressure in the SH and DOCA-salt models of hypertension (18, 22, 26, 28–30, 39). The antihypertensive effect of GABA stimulation appears to result from a decrease in peripheral sympathetic nerve activity (28, 39) as well as a reduction in adrenal nerve activity and decrease in plasma Epi independent of changes in plasma NE (22, 39). These findings suggest that an increased sympathoadrenal function observed in hypertensive animals may be the result of an attenuation of GABAergic activity in the central nervous system.

The possible site(s) at which GABA function may be altered in hypertensive animals have not been fully defined. However, decreases in hypothalamic GABA function may contribute to the hypertensive process. Although the hypothalamus contains a relatively high concentration of GABA (8, 40) in normotensive animals, hypothalamic GABA content is reduced in animal models of hypertension (4, 12). In addition, GABA receptor number and affinity are also decreased in hypertension (4, 12, 17). The precise hypothalamic site involved remains to be defined. Although GABA release
is not significantly different in the posterodorsal hypothalamus of Wistar-Kyoto (WKY) and SH rats (38), microinjection of muscimol directly into the DMH (then described as posterior hypothalamic area) induced a greater depressor response in SH rats than in normotensive rats (42). However, the injection of BMI into this area of the hypothalamus failed to elicit the increase in blood pressure and heart rate in SH and WKY rats (42). More recently, administration of a GABA synthesis inhibitor in the posterior hypothalamus (dorsal to the DMH) failed to elicit the increase in blood pressure in SH rats that was observed in normotensive rats (17). Thus, although a reduced GABAergic function in the more dorsal portion of the posterior hypothalamus may contribute to an increased sympathetic outflow in hypertension, the dorsomedial region of the hypothalamus has not been excluded as a possible site.

The PVN has received considerable attention as an important area involved in sodium-dependent hypertension. Indeed, lesions of the PVN attenuate the development and reverse the elevated pressure observed in animals with sodium-dependent hypertension (10, 14, 23). Moreover, inhibition of GABA activity in the PVN elicits an increase in sympathoadrenal outflow, blood pressure, and heart rate (20). The results of the present study confirm these findings. In sham-operated normotensive rats, administration of BMI caused increases in arterial pressure and heart rate that were accompanied by two- and threefold increases in plasma NE and Epi, respectively. Thus GABA appears to exert a tonic inhibitory effect on sympathetic outflow from the PVN.

The present study was undertaken to test the hypothesis that a decrease in GABA function in the PVN could contribute to renal wrap hypertension. One prediction of this hypothesis is that the responses to BMI should be reduced if there is a preexisting reduction in GABA tone in the hypertensive animals. Consistent with this prediction, we observed that arterial pressure did not increase in the hypertensive animals, whereas the plasma NE and Epi responses were reduced by 75 and 68%, respectively. Thus the sympathoadrenal responses to BMI administration into the PVN resulting in vasoconstriction were markedly blunted in the animals subjected to renal wrap hypertension. Nevertheless, it was clear that BMI was exerting an effect in the PVN of hypertensive rats since the behavioral and heart rate responses to BMI were similar to those of the control rats. The presence of the BMI-induced tachycardia, despite a significant reduction in the vascular sympathetic nervous system responses, suggested that pathways regulating heart rate remained intact. One interpretation of these data is that sympathetic activation of the heart is unaffected in hypertensive animals. Alternatively, impairment of sympathoadrenal excitatory pathways in hypertensive rats may permit the predominance of a vagal pathway that has previously been demonstrated to receive input from a site in the rostral hypothalamus (7, 27). Thus the BMI-evoked tachycardia observed in the present study may reflect withdrawal of cardiac vagal tone. Either of these explanations implies a selective deficit in vascular sympa-
The paraventricular nucleus (PVN) responses to muscimol administration into the paraventricular nucleus.

Table 1. Mean arterial pressure and heart rate responses to muscimol administration into the paraventricular nucleus

<table>
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<tr>
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<th>Mean Arterial Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Initial</td>
</tr>
<tr>
<td>Sham</td>
<td>124 ± 2</td>
<td>122 ± 2</td>
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<tr>
<td>Wrap</td>
<td>150 ± 5*</td>
<td>145 ± 4†</td>
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Values are means ± SE; n = 5 sham-operated animals and 8 renal-wrapped animals. *Significant differences between groups. †Significant difference from control.

GABAergic modulation of sympathetic nervous system responsiveness in renal wrap hypertension.

Studies from other laboratories have demonstrated that other sites within the hypothalamus such as the dorsomedial and posterior hypothalamic areas are also important sites for GABAergic modulation of sympathetic outflow (6, 33, 34, 35). It has been suggested that some of the effects ascribed to PVN microinjections may in fact be due to diffusion of drug to these distal sites (6). However, several observations suggest that the responses that we observed were mediated primarily via activation of PVN neurons and not via diffusion to other sites. First, the responses to BMI bolus injections have a short latency to onset (30–60 s), which is not consistent with extensive diffusion. Second, the amount of drug diffusing to these sites would have been relatively small. On the basis of our autoradiographic study of the distribution of radiolabeled BMI injected into the PVN, we would estimate that ~3% of the original dose would reach a site such as the DMH, which is ~1.0 mm away from the placement of our injectors. Thus ~1.5 pmol (3% of 50 pmol injection) would reach the DMH. This amount is below that shown to be necessary to elicit cardiovascular responses from the DMH (35). The behavioral responses elicited from these sites differ. We consistently observed a stereotypical behavior consisting predominately of facial grooming when BMI is injected into the PVN. In contrast, BMI injections into the DMH or posterior hypothalamus are associated with locomotor escape behavior (33). Collectively, these observations strongly suggest that the responses we observed were mediated primarily via activation of neurons in the region of the PVN. Thus, both the PVN and DMH are distinct and important sites at which GABA exerts control over sympathetic outflow.

A reduction in GABAergic tone in the PVN of hypertensive rats could arise as a result of either a presynaptic or postsynaptic action. Presynaptically, a decrease in GABA release or GABA cell number could lead to a decrease in GABAergic input. Few studies have directly addressed this possibility. However, GABA terminal density is reportedly increased in the PVN after 4 wk of DOCA-salt treatment (44). If endogenous GABA release is reduced at a presynaptic site, then the PVN may become more sensitive to agonist stimulation. In normotensive animals, stimulation of the PVN with GABA agonists has little effect on blood pressure due to a maximal tonic inhibition of sympathetic outflow by the neurotransmitter. Administration of muscimol to hypertensive animals elicited significant decreases in blood pressure over a period of ~20 min. However, the 9-mmHg fall in arterial pressure is modest relative to the arterial pressure difference between normotensive and hypertensive rats.

An alternative explanation for the attenuated response to bicuculline is a reduction in receptor number or affinity. Indeed, receptor binding studies in hypothalamic tissue taken from SH rats indicate a decrease in the number of GABA<sub>A</sub> receptors. Hambley et al. (12) showed a decrease in [<sup>3</sup>H]muscimol binding in 11- to 16-wk-old SH rats, but not in 4- to 5-wk-old animals. Binding affinity was not altered at either age. These results were confirmed in a later study (4), which demonstrated a reduction in the number of hypothalamic binding sites in 8- and 14-wk-old SH rats. The location of the reduced GABA binding in the hypothalamus has been localized to the PVN in 12-wk-old SH rats; however, the other hypothalamic sites were not examined (17).

Intravenous infusions of nitroprusside were used to assess sympathetic nervous system responsiveness in the normotensive and hypertensive rats. With equivalent decreases in blood pressure, similar increases in heart rate occurred in both groups, suggesting that cardiac baroreflex function was not impaired in the hypertensive animals. However, the increases in NE and Epi were significantly attenuated in the hypertensive animals, suggesting some attenuation of baroreflex-mediated release of NE. Nevertheless, the hypotension-induced release of NE (~115 pg/ml) was twice as great as that induced by BMI stimulation of the PVN (50 pg/ml) in the renal-wrapped rats. In contrast, these stimuli were equally effective at inducing NE release in the sham-operated rats. These data suggest that, although there may be some impairment of baroreflex-mediated NE release in the hypertensive rats, there is an even greater attenuation of PVN-mediated NE release. These data are consistent with the overall hypothesis that there is a preexisting deficit in PVN GABAergic tone in the renal wrap hypertensive rats.

Perspectives

One-kidney, figure eight renal wrap hypertension is a sodium-dependent model of increased arterial pressure that is sustained by an elevated sympathetic nervous system function (13). Although previous work has demonstrated an involvement of the PVN in this model of hypertension (14), the present study extends that work and indicates that the increase in arterial pressure is associated with an alteration in the function of at least one neurotransmitter. The GABAergic system in the PVN normally provides a tonic inhibition on the sympathoadrenal nervous system (20). However, in renal wrap hypertensive rats the inhibitory actions of GABA are diminished, supporting a role for the PVN in the pathways involved in the activation of the sympathetic nervous system. It is uncertain from these studies whether the change in GABA function is a cause of
the hypertension or a result of the increase in blood pressure.

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