Sympathoinhibitory Effects of Central Nifedipine in Spontaneously Hypertensive Rats on High Versus Regular Sodium Intake

Bing S. Huang, Frans H.H. Leenen

Abstract—The putative central sympathoinhibitory actions of the dihydropyridine calcium antagonist nifedipine and the effect of dietary sodium on these actions were investigated in spontaneously hypertensive rats (SHR). Regular or high dietary salt was administered from 4 to 8 weeks of age. At 8 weeks, blood pressure (BP), heart rate (HR), and renal sympathetic nerve activity were recorded in conscious rats at rest as well as in response to intravenous (50 μg/kg) and intracerebroventricular (5 and 50 μg/kg) injections of nifedipine and intracerebroventricular injections of vehicle. Resting mean arterial pressure was higher in SHR on high versus regular salt (159 ± 3 versus 135 ± 4 mm Hg; P < 0.05). Nifedipine administered intracerebroventricularly decreased BP as well as renal sympathetic nerve activity and HR in a dose-related manner. The responses reached their peak at 3 to 5 minutes and lasted ≈30 minutes. Peak decreases in BP, renal sympathetic nerve activity, and HR in response to both doses of nifedipine were significantly larger in SHR on high versus regular salt. Nifedipine administered intravenously also decreased BP but, in contrast, caused (reflex) increases in renal sympathetic nerve activity and HR. On both diets, intracerebroventricular vehicle did not affect mean arterial pressure, renal sympathetic nerve activity, or HR. These data indicate that in contrast to its peripheral vasodilator effect, centrally administered nifedipine may decrease sympathetic outflow and therefore BP and HR. The enhanced sympathoinhibitory and depressor responses to nifedipine in SHR on high versus regular salt suggest that the sympathetic hyperactivity induced by high salt intake is dependent on neuronal calcium influx via L-type channels. (Hypertension. 1999;33:32-35.)

Key Words: nifedipine ■ dihydropyridines ■ nervous system, sympathetic ■ hypertension ■ rats, inbred SHR ■ sodium, dietary

An increase in Ca\(^{2+}\) influx triggers a variety of cellular functions, such as increased cardiac and smooth muscle contractility, endocrine gland secretion, and neurotransmitter release. Dihydropyridines such as nifedipine inhibit Ca\(^{2+}\) influx through voltage-sensitive calcium channels. The effects of calcium antagonists on sympathetic activity have thus far been related mainly to reflex responses after rapid lowering of blood pressure (BP) induced by rapidly acting calcium antagonists and perhaps presynaptic effects. Although receptors for calcium antagonists as well as calcium antagonist–sensitive calcium channels have been demonstrated in the brain, little is known regarding direct central effects of Ca\(^{2+}\) antagonists on sympathetic activity and central control of circulation.

In vitro, dihydropyridine-sensitive Ca\(^{2+}\) channels exist in rat hypothalamus. The stimulus-induced high-frequency activity in brain cells is carried by L-type but not T-type Ca\(^{2+}\) channels, and a dihydropyridine such as nifedipine is a potent L- but not T-type channel antagonist. Although centrally administered calcium antagonists have been reported to lower BP as well as heart rate (HR) in anesthetized rats, thus far no studies have been reported regarding direct central effects of dihydropyridines on efferent sympathetic nerve activity, BP, and HR in conscious rats.

Spontaneously hypertensive rats (SHR) are characterized by sympathetic hyperactivity, and high dietary sodium further increases sympathetic outflow and the development of hypertension. In SHR or humans with essential hypertension, the antihypertensive effects of oral nifedipine are enhanced by high salt intake. In the present study we therefore examined in conscious SHR whether acute intracerebroventricular injection of nifedipine can elicit sympathoinhibitory, depressor, and bradycardic responses and whether the extent of these responses differs in SHR on high versus regular dietary sodium. The results show that in contrast to its peripheral effects, in SHR intracerebroventricular nifedipine decreases renal sympathetic nerve activity (RSNA), BP, and HR in parallel and that these responses are enhanced by high dietary salt intake.

Methods

Male SHR were purchased from Taconic Farms (Germantown, NY) at 3 to 4 weeks of age. They were housed on a 12-hour light/dark cycle.
cycle and fed regular rat chow and tap water. After a 3-day acclimatization period, they were allocated to either a regular or high salt diet (containing 120 or 1370 μmol sodium per gram food; Harlan Sprague-Dawley) for 4 weeks. Final hemodynamic assessments were done at the end of the dietary period. All procedures were performed according to the guidelines of the University of Ottawa Animal Care Committee.

Approximately 1 week before the final assessments, under pentobarbital sodium anesthesia (65 mg/kg IP), a 23-gauge, stainless steel guide cannula was implanted and fixed to the skull (coordinates: 0.4 mm posterior and 1.3 mm lateral to bregma) for intracerebroventricular injection to the right lateral ventricle. Penicillin G (30 000 IU, Derapen, Ayerst Laboratory) was given after the surgery. At 8 weeks of age, in the early morning, under halothane anesthesia, catheters were inserted into the right femoral artery and vein. With supplemental methohexital sodium (30 mg/kg IV, Brevital, Eli Lilly Canada), a pair of silver electrodes (A-M System) was placed around the left renal nerve through a flank incision and secured with silicone rubber (SilGel 604, Wacker). The catheters and electrodes were tunneled and exteriorized on the back of the neck.

Four hours after recovery from the anesthesia, the free-moving rat was placed in the original cage without restriction. The intra-arterial catheter and electrodes were linked to a Grass polygraph (model 7E), a Grass 7P44 tachogram, and a Grass P511 amplifier for recordings of BP, HR, and RSNA throughout the experiment. RSNA (spikes per second) was counted through a nerve traffic analyzer (model 706C, University of Iowa Bioengineering). The actual RSNA was determined by subtraction of noise from the total activity. The noise was recorded 20 minutes after the rats had been killed.

Thirty minutes were allowed for stabilization before the start of protocol, and then resting MAP, HR, and RSNA were recorded for 5 minutes. The following procedures were performed in a random order: (1) nifedipine 5 μg/kg in 5 μL vehicle ICV; (2) nifedipine 50 μg/kg in 5 μL vehicle ICV; (3) nifedipine 30 μg/kg in 5 μL vehicle IV; (4) 5 μL vehicle ICV; and (5) 5 μL vehicle IV. The next injection was not given until responses to the previous injection had subsided and the rat had rested for an additional 15 minutes. For intracerebroventricular injection, a Hamilton microsyringe (20-μL volume) was used, connected to a 26-gauge L-shaped stainless steel needle via polyethylene tubing. Both polyethylene tubing and needle were filled with the solutions for injection. The longer arm of the needle was inserted into the intracerebroventricular guide cannula so that its tip protruded into the lateral ventricle during the injections. The rate of intracerebroventricular injections was 10 μL/min. The vehicle solvent for nifedipine consisted of 96% ethanol (20%), polyethylene glycol 400 (30%), and artificial cerebrospinal fluid (50%). The solution was prepared freshly in a darkened room every other day and kept in an opaque bottle. During the injection, the syringe was wrapped with aluminum foil and the polyethylene tubing was painted dark.

Changes in RSNA are presented as percentage of resting values. Maximum responses of MAP, RSNA, and HR were expressed as changes from resting values. Statistical analysis was performed by means of paired or unpaired t test or 1-way ANOVA. Statistical significance was defined as P<0.05.

Results

At 8 weeks of age, resting MAP was significantly higher in SHR on high versus regular sodium (159±3 versus 135±4 mm Hg; P<0.05). Resting HR (422±13 versus 415±14 bpm) and body weight (not shown) did not differ significantly between rats on high versus regular salt.

Responses to Intravenous Nifedipine

In SHR on either high or regular sodium, intravenous vehicle did not change MAP, HR, or RSNA (Figure 1). In both groups, ≈30 seconds after 50 μg/kg IV nifedipine, MAP started to decline and reached its lowest level at 5 minutes after the injection. MAP then gradually returned to resting levels within the next 20 minutes. RSNA and HR started to rise within 1 minute after the injection and reached peak levels at 5 minutes (Figure 1). They then declined and returned to resting levels within the next 20 minutes. The peak responses in MAP, RSNA, and HR (Figure 2), as well as the time courses (Figure 1) of the responses, were similar in SHR on high versus regular sodium.

Responses to Intracerebroventricular Nifedipine

Administration of vehicle intracerebroventricularly did not change resting MAP, RSNA, and HR significantly (Figure 3).

Figure 1. Changes in MAP, RSNA, and HR in response to intravenous injection of 50 μg/kg nifedipine or vehicle in SHR on high sodium intake (HNa) or regular sodium intake (RNa). Values are mean±SEM (n=8 for both groups). MAP and HR responses at 5 and 10 minutes and RSNA responses at 2, 5, 10, and 15 minutes after the injection of nifedipine were significantly different from basal values or values at corresponding time points after injection of vehicle. Responses were similar in rats on high versus regular salt intake.

Figure 2. Maximal changes in MAP, RSNA, and HR in response to intracerebroventricular injection of 5 and 50 μg/kg nifedipine or intravenous injection of 50 μg/kg nifedipine in SHR on high sodium intake (HNa) or regular sodium intake (RNa). Values are mean±SEM (n=8 for both). a, P<0.05 vs regular sodium intake with 5 μg/kg ICV. All responses, except HR response to 5 μg/kg ICV, were significantly different from resting values.
In SHR on either regular or high sodium, nifedipine administered intracerebroventricularly caused dose-related decreases in MAP as well as in HR and RSNA. Significant decreases occurred within one half to 1 minute after the intracerebroventricular injection, lowest levels were reached within the next 2 to 4 minutes, and responses then gradually disappeared within the next ~20 to 35 minutes. The time course of responses was similar in rats on high versus regular sodium (Figure 3). In contrast, the extent of decreases of MAP, RSNA, and HR after intracerebroventricular nifedipine was significantly larger in SHR on high versus regular sodium (Figure 2). Peak decreases in MAP by nifedipine were 11±2 versus 5±1 mm Hg for 5 μg/kg, and 27±3 versus 16±2 mm Hg for 50 μg/kg, in SHR on high versus regular sodium, respectively (P<0.005 for both). Similarly, in SHR on high versus regular sodium, peak decreases in RSNA and HR by nifedipine were 13±2% versus 7±1% and 8±1 versus 4±1 bpm for 5 μg/kg and were 30±2% versus 19±2% and 21±2 versus 14±2 bpm for 50 μg/kg, respectively (P<0.05 for all).

Discussion

The present study provides 2 major new findings. First, in contrast to its peripheral effects, centrally administered nifedipine decreases RSNA and HR in conscious SHR. Second, these sympathoinhibitory effects of nifedipine are enhanced in SHR on high versus regular sodium intake.

Dihydropyridine-sensitive, low-voltage-activated calcium channels have been demonstrated in rat brain neurons freshly isolated from regions such as the ventromedial hypothalamus. The role of the different types of calcium channels in neuronal synaptic transmission has not been clarified. In rat hippocampal cells, during neuronal activation postsynaptic or presynaptic Ca2+ entry involves the activation of the P/Q-, L-, or N-type Ca2+ channels but not the T-type Ca2+ channels. Unless dissolved in certain solvents such as dimethylsulfoxide, nifedipine blocks only L-type channels and has no effects on T-type channels in neuronal cells. In the present study nifedipine was dissolved in a vehicle containing no dimethylsulfoxide. The dose-related sympathoinhibitory responses to intracerebroventricular but not intravenous nifedipine are therefore likely due to inhibition of L-type calcium channels in the brain.

Only a few studies in vivo have been reported thus far concerning the hemodynamic effects of centrally administered Ca2+ antagonists in rats. In anesthetized SHR and Wistar-Kyoto rats, intracerebroventricular injection of nifedipine at the same doses as used in the present study decreased BP and HR in a dose-related fashion, whereas the dihydropyridine Ca2+ channel agonist Bay K 8644 administered intracerebroventricularly increased BP and HR. Laurent et al suggested that nifedipine and Bay K 8644 may decrease or increase sympathetic outflow by affecting the discharge rate of vasomotor neurons in the brain. Although the directions of MAP and HR responses of the present versus the aforementioned study are the same, the extent and the time course of responses are different. The use of general anesthesia, which affects central neuronal activity including baroreflex function, as well as the use of 95% ethanol as the vehicle by Laurent et al may contribute to the different results. In Wistar-Kyoto rats under urethane anesthesia, nifedipine, diltiazem, or verapamil administered on the dorsal surface of the brain stem at the obex decreased BP and HR. These responses to the Ca2+ antagonists were not observed in rats pretreated with 6-OH dopamine, suggesting that these responses result from the withdrawal of sympathetic activity. Since these effects of Ca2+ antagonists were markedly attenuated by bilateral electrolytic lesions of the nucleus tractus solitarius and diltiazem injected into the nucleus tractus solitarius decreased BP and HR, the authors suggested that the nucleus tractus solitarius is involved in the withdrawal of sympathetic activity by calcium antagonists. In rats under urethane anesthesia, intracerebroventricular diltiazem decreased BP, HR, and abdominal sympathetic nerve activity. These decreases were attenuated by electric lesions of the anteroventral third ventricle, suggesting the involvement of Ca2+ channels in the anteroventral third ventricle in these responses.

In a previous study we demonstrated that in young SHR modest increases in salt intake for 12 weeks did not exacerbate the development of hypertension but enhanced the hypotensive effect of orally administered nifedipine. Since the hypotensive effect of ganglionic blockade was significantly blunted by nifedipine in SHR on high salt intake, we suggested that high salt potentiates the inhibitory effects of nifedipine on sympathetic activity. The present study provides direct evidence in this respect, showing that the extent of decreases in RSNA, HR, and MAP by nifedipine admin-
istered intracerebroventricularly was significantly larger in SHR on high versus regular salt. The mechanisms by which high salt intake enhances the sympathoinhibitory effects of central nifedipine in SHR have not yet been elucidated. In SHR, high salt intake–induced increase in brain ouabain-like activity mediates sympathetic hyperactivity and exacerbation of hypertension on high salt.11,18 One may speculate that increased brain ouabain-like activity increases neuronal calcium influx, and the latter leads to a sensitization of responses in sympathetic neuronal activity to blockade of calcium channels. Changes in central binding sites for nifedipine may also contribute to the enhanced responses. In adult SHR, high salt intake increases cardiac dihydropyridine binding sites.19 Further studies are needed to examine whether in SHR high salt intake also increases brain binding sites for dihydropyridines, leading to enhanced responses to intracerebroventricular nifedipine.

Whether a central sympathoinhibitory effect occurs after peripheral administration of a dihydropyridine cannot be determined from the present study. However, in SHR long-term oral treatment with nisolipine significantly decreased BP associated with decreased cardiac sympathetic activity, as assessed by cardiac norepinephrine turnover rate.20 Similarly, in SHR, chronic treatment with amlodipine or manidipine lowered plasma norepinephrine, whereas hydralazine caused a further increase.21 Such decreases in peripheral parameters of sympathetic activity may, at least in part, relate to central effects of the dihydropyridines. The extent of such central effects with oral treatment is likely related to the degree of lipophilicity, which would determine speed and extent of crossing of the blood-brain barrier.

In conclusion, the present study demonstrates that in contrast to its peripheral effects, the 1,4-dihydropyridine nifedipine administered intracerebroventricularly induces parallel decreases in BP, HR, and RSNA in conscious SHR. In SHR on high versus regular sodium intake, increased resting BP is associated with enhanced inhibitory BP, HR, and RSNA responses to intracerebroventricular nifedipine. These results indicate that calcium influx via L-type calcium channels in the central nervous system plays an important role in the regulation of sympathetic outflow and arterial BP in SHR. In young SHR, sympathetic hyperactivity and exacerbation of hypertension by high salt intake may involve changes in properties of the central calcium channels, as reflected in enhanced responsiveness to intracerebroventricularly administered nifedipine.

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