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Enhanced Antinociception of Clonidine in Spontaneously Hypertensive Rats Involves a Presynaptic Noradrenergic Mechanism

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WANG, Y., C. Y. CHENG, J. Y. WANG AND J. C. LIN. *Enhanced antinociception of clonidine in spontaneously hypertensive rats involves a presynaptic noradrenergic mechanism.* PHARMACOL BIOCHEM BEHAV **59**(1) 109–114, 1998.— We and others previously reported that the antinociceptive effect of clonidine, measured by the hot plate method, was greater in spontaneously hypertensive rats (SHRs) than in Wistar–Kyoto rats (WKYs). In the present study, we found that the difference in clonidine-induced analgesia between these two strains was abolished after lesioning the presynaptic noradrenergic neurons with N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4). Previous studies indicated that clonidine increases tissue norepinephrine (NE) content by inhibiting NE release. We found that the basal NE concentration in locus coeruleus (LC), as measured by HPLC-ECD, was not different between WKYs and SHRs. Systemic application of clonidine (0.69 mg/kg, IP) significantly increased the tissue content of NE in the SHRs, but not in WKYs. Using pressure microinjection and high-speed chronoamperometric recording techniques, we found that local application of KCl to the LC brain slices increased extracellular NE levels in both strains. Perfusion of slices with clonidine (1 μM) selectively attenuated KCl-evoked NE release in SHRs, suggesting that clonidine-induced presynapitc inhibition is more effective in SHRs than in WKYs. In conclusion, our data indicate that SHRs possess augmented sensitivity to clonidine to inhibit presynaptic NE release, which may be responsible for the enhanced antinociceptive effect of clonidine in this strain. © 1998 Elsevier Science Inc.

Clonidine Analgesia Norepinephrine Locus coeruleus Voltammetry SHR

CENTRAL noradrenergic pathways are involved in modulation of analgesic responses. Electrical stimulation of locus coeruleus (LC) extended the latency to hot plate stimulation in rats (19,42). This analgesic effect was antagonized by intrathecal administration of the α_2 antagonist yohimbine. Similarly, systemic or intrathecal injection of the α_2 agonist clonidine prolonged the latency to the nociceptive stimuli measured by hot plate, tail flick (17,31,36) or formalin tests (14) and suppressed electrophysiological activation of c fibers (35). The clonidine-mediated antinociceptive effects were antagonized by intrathecal administration of yohimbine (43). These data indicate that stimulation of LC or administration of clonidine induces an antinociceptive effect through activation of central α_2 receptors (26,29).

It has been reported that the antinociceptive effect of clonidine (0.69 mg/kg) was greater in the spontaneous hypertensive rats (SHRs) than in Wistar–Kyoto rats (WKYs). The enhanced antinociceptive effect of clonidine in SHRs is not directly related to blood pressure (17) or pharmacokinetic factors (20). Peripheral administration of radiolabeled clonidine revealed no difference between SHRs and WKYs in the levels of clonidine in the central nervous system (CNS) (20). On the other hand, central noradrenergic activity is differentially regulated between SHRs and WKYs (5,6,12). SHRs have a higher norepinephrine (NE) content in the nucleus tractus solitarius and posterior hypothalamic area 4 to 11 weeks after birth (44). The high-affinity uptake and the release of NE are potentiated in the SHRs, compared to the WKYs (9,15,23).

Clonidine binds to the postsynaptic extrajunctional and presynaptic α_2 receptors. Depletion of presynapitc NE stores with reserpine (22) or lesioning the central monoaminergic neurons with 6-OHDA did not abolish clonidine-elicited analgesia (10,27,31), suggesting that the direct antinociceptive action of clonidine is mainly mediated through postsynaptic α_2 mechanisms. Because clonidine also regulates NE release pre-

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synaptically, it is possible that the postsynaptic effect of clonidine can be modulated by the presynaptically released NE.

In the present study, we used N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) and a low dose of methamphetamine (MA) to investigate the mechanism of the enhanced antinociceptive effect of clonidine in SHRs. Previous studies indicate that DSP4 selectively reduces up to 76% of central NE content without attenuating dopamine and serotonin concentrations (1,13,39). We also measured the tissue NE content and the extracellular NE levels in the LC using HPLC-ECD and voltammetry. Our data suggest that SHRs have a higher sensitivity to clonidine to inhibit presynaptic NE release, which may contribute to the hypersensitivity in antinociception.

METHOD

Adult SHRs (247 ± 8 g), and age-matched (12–15 weeks old) Wkys (265 ± 10 g) were used in these experiments. All animals were housed under identical conditions of temperature and 12 L:12 D cycle, and were allowed access to food and water ad lib.

Measurement of Arterial Pressure

The systolic arterial pressure was measured noninvasively from the pulse of the tail of conscious rats by tail-cuff plethysmography (Iitc, Inc., Woodland, Hills, CA., MOD 59 Blood Pressure Meter/Amplifier). Rats were trained to sit quietly for 3–5 min in a dark restraining cage before measurement. Room temperature was kept between 26–28°C. Blood pressure was recorded as the mean of five separate determinations.

Assessment of Nociceptive Responses

Sensitivity to pain was determined by the hot-plate test. Animals were placed into an observation chamber consisting of clear Plexiglas wall and metal floor that was maintained at 52.0 ± 0.5°C. The day before testing, animals were placed on the nonfunctioning hot plate for 1 min. The rats were brought to the test room 2 h before testing and the ambient temperature during testing was 27 ± 1°C. The latency of licking a hind paw or jumping off (vigorous lifting of both rear paws) the plate was taken as the measure of nociceptive threshold (18,20,37). Cutoff time was set at 40 s to prevent tissue injury. Each animal was studied once. Response latencies were normalized to percentage of maximal possible effect (% MPE) as follows (17):

$$\%\ \text{MPE} = \frac{\text{Postdrug latency} - \text{Predrug latency}}{\text{Cutoff time} - \text{Baseline latency}} \times 100\%$$

Measurement of Tissue Levels of NE in LC

Animals were sacrificed by decapitation. After removal of the cerebellum, a coronal slicer (2 mm in width) was placed vertically with rostral end against caudal midbrain. LC slices were quickly removed and kept at −70°C until analysis (11). For analysis, tissue samples were weighed and homogenized in 0.1 N perchloric acid using a Polytron homogenizer. After 30 min of incubation on ice, the samples were centrifuged for 30 min at 32,000 × g at 4°C; the supernatants were assayed for NE by high-performance liquid chromatography coupled with electrochemical detection (HPLC-ECD) (21). The HPLC apparatus consisted of a Kratos solvent delivery system (Spectroflow 400) delivering the mobile phase at a constant flow of 0.8 ml/min to a reversed-phase column (25 0.4 cm Supersphere, 4 μm, E. Merck, Darmstadt, Germany) fitted with a guard column (3 0.4 cm, dry packed, Lichrosorb C, 30 μm, E. Merck, Darmstardt, Germany). Electrochemical detection was performed using a Bioanalytical System LC4B amperometric detector cell with a glassy carbon electrode. Applied potential was maintained at 0.5 V vs. an Ag-AgCl reference electrode.

Measurement of KCl-Evoked NE Release in LC Slices

After brief ether anesthesia, rats of either strain were decapitated and the midbrain was removed and placed in cold, modified Earle's medium (see below). The LC slices were cut with a vibratome in 400 μm thickness. The slices were placed in a beaker filled with modified Earle's medium, continuously bubbled with 95% O_2/5% CO_2 and maintained at 30°C, and then transferred one at a time to an immersion microperfusion chamber for recording. Modified Earle's medium equilibrated with 95% O_2/5% CO_2 was allowed to flow continuously through the microperfusion chamber, superfusing the tissue at the rate of 2.7–3.0 ml/min, and maintained at 35°C.

Extracellular NE concentration was measured by a microcomputer-controlled chronoamperometric apparatus (IVEC-10, Medical Systems Corp., Greenvale, NY). The recordings were taken at rates of 10 Hz continuously using Nafion-coated (5% solution, Aldrich Chemical Co., Milwaukee, WI) carbon-fiber working electrodes (7,41). These electrodes have been shown to be highly sensitive for catecholamines (7). An oxidation potential of 0.55 V for 50 ms (square-wave pulses), relative to a Ag/AgCl reference electrode, was applied at a rate of 10 Hz. The resulting oxidation current was integrated during the last 80% of the pulse. The current generated during the reduction (50 ms) of the oxidized electroactive species was digitized in the same manner when the potential dropped back to its resting level (0 V). The ratios of reduction-to-oxidation currents, at the peak of the oxidation signal, were used as an index to qualitatively identify the compound measured as NE (2,33). The linearity and sensitivity of all electrodes used for the in situ experiments were determined using NE standard solutions in vitro ranging from 0 to 10 μM. All solutions were prepared in 0.1 M pH 7.4 phosphate-buffered saline, which also contained 250 μM ascorbic acid to mimic brain extracellular levels of this potential contaminant of the electrochemical recordings (32). Calibration curves for NE and NE-to-ascorbic acid selectivity ratios were determined for all electrodes prior to their use. Only electrodes exhibiting highly linear responses ($r > 0.997$) and selectivity (>500:1, compared with ascorbic acid) to NE were used.

The release of NE was measured by the changes in extracellular NE concentration after microejection of KCl into the LC brain slice. KCl (90 mM, 200–400 nl) was locally applied through micropipettes (8). The working electrode and the micropipette were mounted together with sticky wax (Kerr Inc., Sybron, CA); tips were separated by 100–150 μm. The electrode/pipette assembly was lowered into the LC. Local application of KCl from the micropipettes was performed by pressure ejection using a pneumatic pump (PPM-2, Medical Systems Corp., Great Neck, NY). The pressure used for microinjection was 3–15 psi. The duration of injection was 1–4 s. The ejected volumes were monitored by observing the changes of fluid meniscus in the pipette through a stereoscopic microscope.

Drugs and Chemicals

Earle's balanced salt solution (Gibco Laboratories, Grand Island, NY) was modified to give final concentrations of the following compounds: 1.36 mM $CaCl_2$, 5.4 mM KCl, 0.8 mM

$MgSO_4$, 116 mM NaCl, 0.9 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 10 mM D-glucose, and was saturated with 95 O_2/5% CO_2 (−)Norepinephrine hydrochloride (NE), methamphetamine hydrochloride, and clonidine hydrochloride were purchased from the Sigma Chemical Company, St. Louis, MO. Sodium dihydrogen phosphate, EDTA and HPLC grade chemicals and solvents were obtained from Merck (Darmstadt, Germany). KCl solution used for pressure microejection was made in a pH 7.2–7.4 cocktail containing 90 mM KCl, 2.5 mM $CaCl_2$, and 59 mM NaCl. Methamphetamine was injected systemically at a dose of 0.01 mg/kg. Clonidine was injected at the dose of 0.69 mg/kg (IP) 30 min before the hot plate test. N-chloroethyl-N-ethyl-2-bromobenzylamine hydrochloride (DSP4) was purchased from the Research Biochemicals International (Natrick, MA) and was made fresh in distilled water, and two injections were administered IP at a dose of 50 mg/kg 19 and 14 days prior to sacrifice (39,40).

Statistics

Results were expressed as mean ± standard errors of the mean (SEM). For statistical analysis of data, Student's *t*-test or two-way ANOVA was used, as appropriate. If the ANOVA indicated overall significance, Newman–Keuls post hoc analyses were performed to determine significance of between-group means.

RESULTS

A total of 38 WKYs and 40 SHRs were used for measuring tail blood pressure. Baseline systolic blood pressure in the SHRs was significantly higher than that of the WKYs (175.9 ± 2.2 mmHg vs. 126.1 ± 2.7 mmHg, respectively; $p < 0.005$, *t*-test).

Antinociceptive Effect of Clonidine in SHRs and WKYs

The mean baseline latencies of the hot-plate test showed no difference between the SHRs (9.1 ± 0.4 s, $n = 40$) and WKYs (9.7 ± 0.4 s, $n = 38$, $p > 0.2$). Systemic injection of clonidine significantly prolonged the latencies in both strains (WKYs = 23.3 ± 1.3 s, SHRs = 29.7 ± 1.2 s). The antinociceptive effect of clonidine (0.69 mg/kg, IP) as measured by % MPE, taken 30 min after injection, was higher in the SHRs than in the WKY controls (SHRs: 66.8 ± 3.8% vs. WKYs: 41.6 ± 5.1%, $p < 0.005$, *t*-test).

Effects of DSP4 Treatment Upon the Antinociceptive Effect of Clonidine in SHRs and WKYs

Eight WKYs and seven SHRs were lesioned with systemic DSP4 injections. Previous studies indicated that systemic injection of DSP4 only irreversibly lesioned the central, but not the peripheral, noradrenergic neurons, especially the terminals (13,28). Consistent with these findings, we also found that arterial pressure taken 30 days after DSP4 lesioning was not altered compared to that taken before lesioning (WKYs: before lesioning 123.5 ± 3.3 mmHg vs. after lesioning: 121.5 ± 6.6 mmHg, $p > 0.7$ paired *t*-test; SHRs: before lesioning: 165.1 ± 3.1 mmHg vs. after lesioning: 170.4 ± 6.9 mmHg, $p > 0.17$ paired *t*-test).

Thirty days after DSP4 lesioning, animals were tested for their responsiveness to clonidine. We found that % MPE of clonidine in SHRs was not altered by DSP4 (Fig 1, $p > 0.05$, two-way ANOVA). On the other hand, in WKYs, the % MPE of clonidine was potentiated by lesioning ($p < 0.05$, two-way ANOVA + Newman–Keuls test). Before DSP4 lesioning, SHRs were more sensitive to the clonidine-induced analgesia than the WKYs (Fig 1, $p < 0.05$, two-way ANOVA + Newman–Keuls test). After DSP4 lesioning, the difference in clonidine-elicited analgesia between SHRs and WKYs disappeared (Fig. 1, $p > 0.05$, two-way ANOVA).

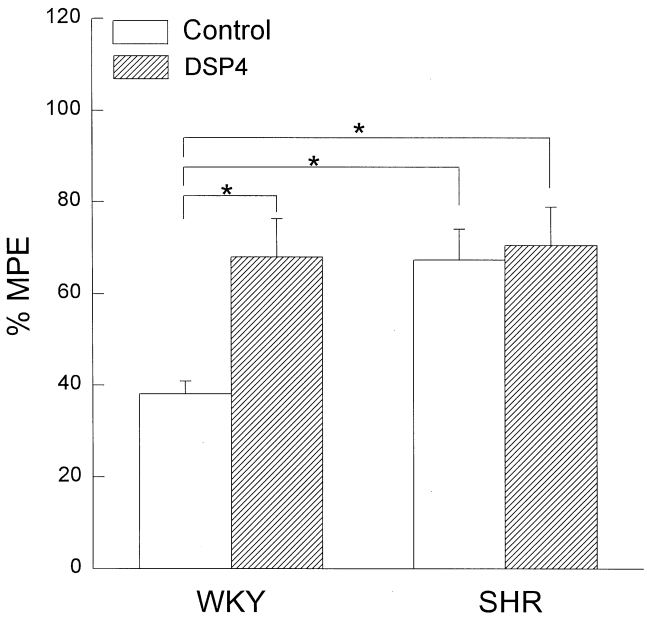


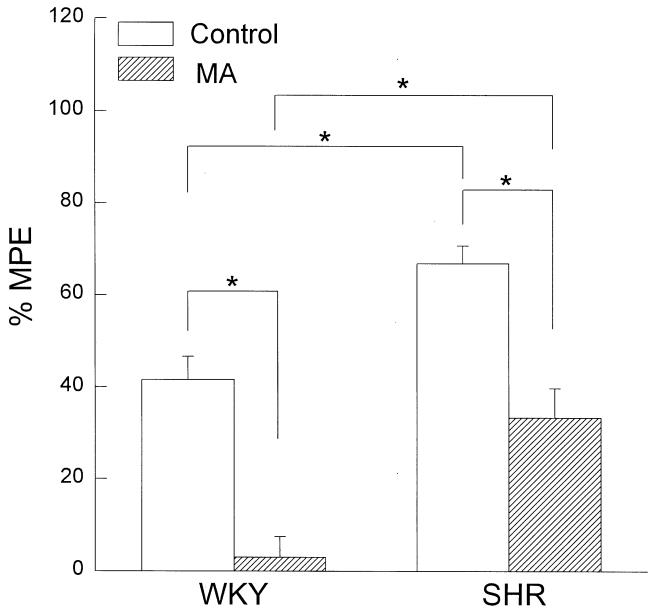
FIG. 1. DSP4 lesioning increased analgesic effect of clonidine in WKYs, but not in SHRs. Animals were tested for their analgesic response (% MPE) to clonidine before (clear bars) and 30 days after DSP4 lesioning (hatched bars). In SHRs, the % MPE after DSP4 lesioning was not different from that before lesioning ($n = 7$). However, in WKYs, the % MPE was potentiated by lesioning ($n = 8$). Note that SHRs have an enhanced antinociceptive effect of clonidine, compared to WKYs, before DSP4 lesioning. The enhanced clonidine response was abolished 30 days after lesioning. (*$p < 0.05$, two-way ANOVA and Newman–Keuls post hoc test.)

Effects of Methamphetamine (MA) Treatment on the Antinociceptive Effect of Clonidine in SHRs and WKYs

We found that subcutaneous administration of a low dose of MA (0.01 mg/kg), an NE releasing agent, did not alter the systemic arterial pressure in either WKYs or and SHRs. However, MA significantly attenuated clonidine-induced antinociception in WKYs (Fig. 2, $p < 0.05$, two-way ANOVA + Newman–Keuls test, without MA, $n = 38$ vs. with MA, $n = 9$) and SHRs (Fig 2, without MA, $n = 40$ vs. with MA: $n = 20$, $p < 0.05$, two-way ANOVA + Newman–Keuls test).

Effect of Clonidine on the Tissue content of NE in LC

We found that the basal NE concentration in LC, as measured by HPLC-ECD, was not different between WKYs and SHRs (WKYs, 275.0 ± 16.3 pmol/g tissue, $n = 10$ vs. SHRs, 296.5 ± 12.7 pmol/g tissue, $n = 9$, $p > 0.3$, *t*-test). Thirty minutes after systemic injection of clonidine (0.69 mg/kg, IP), NE concentration in SHR ($n = 9$) was significantly increased to 351.0 ± 18.8 pmol/g tissue ($p < 0.05$, *t*-test). NE content in WKYs was not affected by clonidine (303.9 ± 20.1 pmol/g tissue, $n = 10$, $p > 0.2$, *t*-test).



Effect of Clonidine on the KCl-Evoked NE Release in the In Vitro LC Slices

We found that KCl-evoked NE release was differentially affected by clonidine in the SHRs and WKYs. Using high-speed chronoamperometric techniques, we found that local application of KCl (90 mM, 346.9 ± 26.1 nl) to LC induced extracellular NE overflow in SHRs (2.58 ± 0.40 μM, $n = 8$). Perfusion of clonidine (1 μM) significantly attenuated this KCl-evoked NE release in the same slices (1.50 ± 0.22 μM, $p < 0.05$, paired t-test). WKYs were less responsive to clonidine. Perfusion of 1 μM clonidine did not alter KCl (90 mM, 267.5 ± 23.6 nl)-evoked NE release from WKY LC slices (1.84 ± 0.26 μM vs. 1.79 ± 0.24 μM, $n = 10$, $p > 0.6$, t-test). Representiative data for the effect of clonidine on KCl-evoked

FIG. 2. Methamphetamine (MA) attenuated the antinociceptive effects of clonidine in SHRs and WKYs. The clonidine-induced analgesic response (% MPE) was compared before (open bar, $n = 39$ in WKYs; $n = 40$ in SHRs) and after MA (hatched bars, $n = 9$ in WKYs; $n = 20$ in SHRs) administration (0.01 mg/kg, SC). MA antagonized clonidine-induced antinociception in both strains. (*$p <$ 0.05, two-way ANOVA and Newman–Keuls post hoc test.)

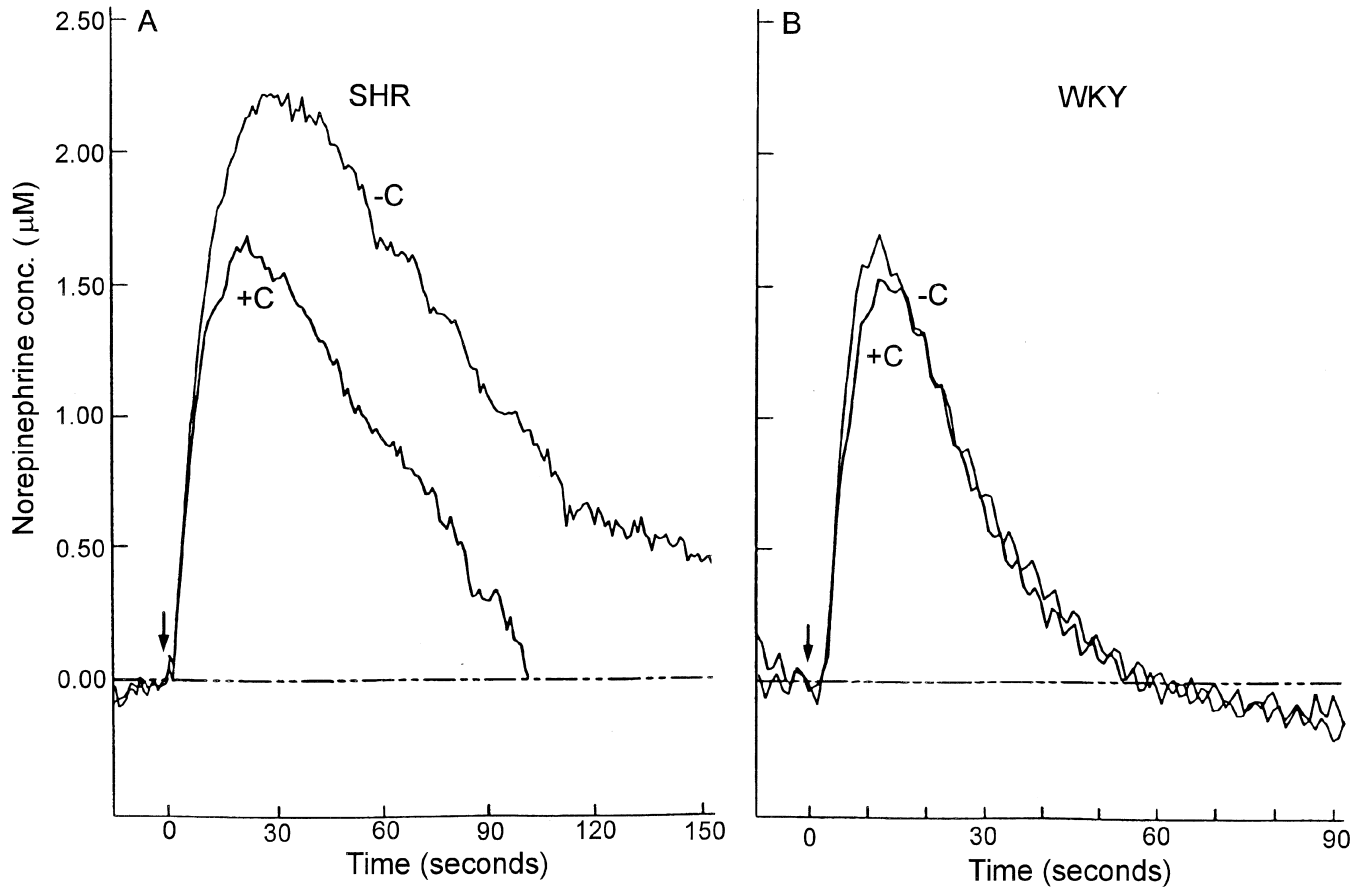


FIG. 3. Clonidine differentially attenuates K^+-evoked NE release in representative LC slices from SHR and WKY rats. Chrono-amperometric recordings demonstrated that direct administration of K^+ (Arrow, 90 mM, 275 nl) to the LC slice induced NE release in the absence of clonidine (−C). Pretreatment with clonidine (+C, 1 μM for 15 min) attenuated K^+-evoked NE release in SHR (A), but not in WKY (B). Data depicted were from a single observation from each rat strain before and after clonidine.

NE release in LC slices from the two rat strains studied are given in Fig. 3.

DISCUSSION

Previous studies indicated clonidine-induced analgesia was produced primarily via activation of postsynaptic α_2 receptors (10,27,31). On the other hand, 6-OHDA treatment or electrolytic lesioning of the LC facilitated clonidine-mediated analgesia in rats (25,31). We also found that clonidine-induced antinociception was potentiated after lesioning the presynaptic NE neurons with DSP4 in WKYs. Application of low dose of methamphetamine (0.01 mg/kg), which facilitates NE release, of which the main effect is to induce synaptic NE release, antagonized the clonidine-induced analgesia. These results suggest that a modulatory mechanism exists in the presynaptic NE neurons that counteracts the postsynaptic analgesic effect of clonidine. Removal of presynaptic inhibition by DSP4 increases the analgesic response to clonidine in the WKYs.

We previously reported that the antinociceptive effect of clonidine was greater in the SHRs than WKYs (17). In this study, we found that lesioning noradrenergic neurons with DSP4 abolished the differential sensitivity of clonidine between WKYs and SHRs. Because lesioning noradrenergic neurons did not increase clonidine binding in the LC, midbrain, pons, and medulla of rats (31,38), the enhanced antinociceptive effect of clonidine is most likely due to a presynaptic mechanism, not from denervation supersensitivity.

Facilitation of synaptic NE release attenuates, while reduction of NE release potentiates, the analgesic effect of clonidine. Because NE release is controlled by presynaptic α_2 receptors, the enhancement of clonidine-induced antinociception in the SHRs may involve inhibition of presynaptic NE release. Previous studies indicated that clonidine increased tissue NE content in the hypothalamus by inhibiting its release (4,34). We and others found that basal content of NE in LC, using HPLC-ECD detection techniques, did not differ between SHRs and WKYs (24,30). However, clonidine significantly increased the tissue content of NE in the SHRs, but not in the WKYs, suggesting that clonidine more potently inhibits basal NE release presynaptically in SHRs. We found that perfusion of 1 μM clonidine selectively diminished KCl-evoked NE release in the LC slices of SHRs, indicating that evoked NE release is also sensitive to exogenously applied clonidine in these hypertensive animals.

Previous studies indicated that clonidine interacts with central α_2 and imidazoline receptors (3). We also recently reported that clonidine modulates the excitatory amino acid-induced hypertensive response through α_2 and imidazoline receptors in the rostral ventrolateral medulla (16). However, pretreatment of yohimbine greatly antagonized the supersensitivity to analgesic responses of clonidine in SHR (20). These data indicate that supersensitivity to analgesic effects of clonidine in SHRs may involve primarily the activation of α_2 receptors in the CNS.

It has been reported that clonidine may induce analgesic responses via both spinal and supraspinal mechanisms. Intrathecal injection of clonidine prolonged the latency to the nociceptive stimuli (14,36). Intrathecal administration of yohimbine antagonized clonidine-mediated antinociceptive effects (36,43). On the other hand, electrical stimulation of supraspinal areas, such as LC, prolonged the latency to hot plate stimulation in rats (19,42). In this study, we found that clonidine differentially regulates NE release in the LC of SHR and WKY. Such a differential response of clonidine at supraspinal level may be responsible for the difference in antinociception in these two strains.

In conclusion, our data suggest that the enhanced antinociceptive effect of clonidine in SHRs may result from the supersensitivity of presynaptic inhibition. Compared to WKYs, SHRs are more sensitive to clonidine-induced presynaptic inhibition, which induces less NE release and results in enhancement of clonidine-induced antinociception. Because clonidine, a commonly used antihypertensive agent, may provide a better analgesic effect to hypertensive animals, this compound should be considered as a potential useful analgesic agent for hypertensive patients.

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