Responsiveness of Locus Ceruleus Neurons in Hypertensive Rats to Vasopressin
KATHLEEN H. BERECEK, HANS-RUDOLF OLPE, AND KARL G. HOFBAUER

SUMMARY We studied the actions of vasopressin administered microiontophoretically onto neurons of the locus ceruleus in rats with deoxycorticosterone-acetate (DOCA)-salt hypertension and in control (normotensive) rats. Rats were studied at 3 days (prehypertensive stage) and 4 to 6 weeks after DOCA-salt treatment (chronic hypertensive stage). Experiments were performed in anesthetized rats using conventional microiontophoretic and single-cell recording techniques. Three days after DOCA-salt administration, the treated rats showed no rise in arterial pressure in comparison with control rats, but 4 to 6 weeks later, the treated rats had significantly greater pressures ($p<0.01$) than controls. Vasopressin administered with currents of 10 to 90 nA for 1 minute produced a current-dependent increase in the firing rate of noradrenergic neurons in all rats. Increases in the firing rate of noradrenergic neurons in DOCA-salt-treated rats, whether in the prehypertensive or the chronic stage, were significantly greater than increases in control rats. These findings indicate that 1) vasopressin can affect neuronal activity in the locus ceruleus and 2) noradrenergic neurons in the locus ceruleus of DOCA-salt-treated rats have an increased responsiveness to the excitatory effects of vasopressin in both prehypertensive and chronic stages of hypertension. (Hypertension 9 [Suppl III]: III-110–III-113, 1987)

KEY WORDS • locus ceruleus • deoxycorticosterone acetate–salt hypertension • vasopressin • vasopressin antagonist

NUMEROUS studies have suggested a primary role for vasopressin in the pathogenesis of deoxycorticosterone acetate (DOCA)–salt hypertension, but its mechanism of action is not clear. Recent evidence has shown that, in addition to its peripheral vasoconstrictor and renal actions, vasopressin has a number of autonomic and behavioral effects mediated by the central nervous system. The vasopressin-synthesizing nuclei (paraventricular and supraoptic) of the hypothalamus send vasopressin-containing projections to a number of central areas involved in cardiovascular regulation, including the locus ceruleus, nucleus tractus solitarii, and the intermediolateral column of the spinal cord. This finding has led to the hypothesis that vasopressin may function as a neurotransmitter in the brain to modulate the activity of specific neuronal systems involved in arterial pressure regulation. Previous observations that intracerebroventricular administration of vasopressin produces long-lasting increases in blood pressure and heart rate support this hypothesis. The recent observation that microinjection of vasopressin directly into the locus ceruleus also produces long-lasting increases in blood pressure and heart rate suggests that this pontine region may be a critical target area for the central effects of vasopressin.

The purpose of our study was to determine the possible interaction between vasopressin and the noradrenergic neurons of the locus ceruleus and to find out whether it is altered in DOCA-salt hypertension. To this end, we studied the actions of vasopressin administered microiontophoretically onto noradrenergic neurons of the locus ceruleus in rats with DOCA-salt hypertension and in control (normotensive) rats. Rats were tested 3 days post–DOCA-salt administration (prehypertensive stage) and 4 to 6 weeks post–DOCA-salt administration (chronic hypertensive stage).
Methods

All experiments were performed in Sprague-Dawley rats weighing 250 to 350 g. DOCA-salt hypertension was induced by a single subcutaneous polymeric silicone implant (Silastic, Dow-Corning, Midland, MI, USA) containing 200 mg/kg DOCA (Doca, Sigma, St. Louis, MO, USA) administered to unilaterally nephrectomized rats given a 0.9% NaCl + 0.2% KCl drinking solution. Control rats were prepared and maintained in the same fashion as the hypertensive rats, with the exception that the silicone implants of the former contained no DOCA. Experiments were performed in rats 3 days after DOCA-salt treatment (pre-hypertensive stage) and 4 to 6 weeks after DOCA-salt treatment (chronic hypertensive stage). In the prehypertensive rats, arterial pressure was measured once immediately prior to experiment to a direct arteriolar catheter implanted in the femoral artery. In rats with chronic DOCA-salt hypertension, systolic arterial pressure was monitored three to four times between the fourth and the sixth week post-DOCA-salt treatment. Systolic arterial pressure in these rats was measured using an indirect tail cuff method while the animals were conscious and restrained and after they had been prewarmed at 37°C for 5 to 10 minutes.

On the day of the experiment, animals were anesthetized with chloral hydrate (400 mg/kg of body weight, i.p.) and placed in the stereotaxic apparatus. The body temperature was maintained between 36.5 and 38°C by a heating cushion. The locus ceruleus was approached stereotaxically from above (1.7 mm posterior to the ear bars; 1.1 mm lateral to midline; 6.5–7.5 mm below the cortical surface). The incisor bar was set 5 mm above the interaural line. Preliminary experiments with iontophoresis of fast green dye showed that neurons sampled were in the confines of the locus ceruleus using these coordinates. Conventional techniques were used to record spontaneous action potentials extracellularly of single noradrenergic neurons in the locus ceruleus and to apply substances iontophoretically. Triple-barreled micropipettes (3–5 μm tip diameter) were used. One barrel contained NaCl (4 M) for recording, the second barrel contained NaCl (4 M) for neutralizing ejection currents, and the third barrel contained arginine vasopressin (0.01 M dissolved in 0.9% NaCl, pH = 5.5; Bachem, Torrance, CA, USA). The peptide was administered as a cation with current strengths from 10 to 90 nA during 1-minute periods. A retaining current of 10 nA was routinely applied. At least 5 minutes were allowed between applications of various doses of the test substance. In preliminary studies there was no evidence for tachyphylaxis with repeated applications of vasopressin, nor was there evidence of an attenuation of the responsiveness of noradrenergic neurons to this agent over time (90 minutes).

Noradrenergic neurons were identified on the basis of several physiological criteria, as described previously. Briefly, the neurons were characterized by a slow and regular firing pattern, their firing frequency ranging from 0.5 to 4 Hz, and the reaction to a painful peripheral stimulus (tail pinch) was a brief phase of strong excitation followed by a long period of firing depression. Two to four neurons were recorded in each rat for periods of 60 minutes each. The same iontophoresis pipette was used for a DOCA-treated and control pair.

Data were expressed as a ratio of the peak increase in firing rate in response to vasopressin (response frequency) to the basal firing rate. In addition, data were expressed as the maximum percentage of change in firing rate in response to vasopressin. Data presented here are mean values ± SEM for the various rat groups. Statistical analysis of blood pressure and basal neuronal firing frequency data was done using one-way analysis of variance and Student's t test. Statistical analysis of the differences in the mean values of DOCA-treated and control rats in response to vasopressin was made using a factorial split-plot design analysis of variance with subsampling. The control versus DOCA effect and the prehypertensive versus chronic hypertensive effect were the two whole-plot factors within which the individual rats (blocks) were nested. The vasopressin dose level with its interactions with the two whole-plot factors were the within-block (subplot) effects. Subsampling (with unequal subsample sizes) was present in that several cells were evaluated for each dose level of vasopressin in each animal. The analysis was done using the General Linear Models Procedure of SAS (Statistical Analysis System Institute, Cary, NC, USA).

Results

At the time of experimentation, DOCA-salt–treated rats at 3 days post-DOCA (prehypertensive stage) showed no significant rise in mean arterial pressure in comparison to control rats (92 ± 5 versus 90 ± 5 mm Hg, respectively); whereas DOCA-salt–treated rats at 4 to 6 weeks post-DOCA had significantly (p < 0.01) greater systolic arterial pressures than controls (185 ± 7 versus 136 ± 4 mm Hg, respectively). Basal firing frequency of noradrenergic neurons from DOCA-salt–treated rats (28 neurons, 0.92 ± 0.05 Hz) was 10% lower than that seen in control rats (28 neurons, 0.83 ± 0.04) at 3 days post-DOCA (prehypertensive stage), but this difference was not statistically significant. Neurons from DOCA-salt–treated rats in the chronic hypertensive stage (33 neurons, 0.98 ± 0.04) showed an average basal firing frequency that was 16% lower than that seen in control rats (34 neurons, 0.82 ± 0.04). This difference was significant (p < 0.01).

Microiontophoretic application of vasopressin to noradrenergic neurons of the locus ceruleus produced excitation of all neurons tested. Ejection of the same amount of current through a 4 M NaCl barrel, or reversal of the polarity of the ejection current in the peptide barrel, did not result in a change in the firing rate of neurons excited by vasopressin. The excitatory response started with a delay of several seconds and
slowly reached its maximum. Recovery occurred gradually over several seconds following cessation of the ejection current.

Noradrenergic neurons from DOCA-salt-treated rats showed greater excitation in response to vasopressin than neurons from control rats. Figure 1 shows the ratios of the response frequency to basal frequency in noradrenergic neurons from prehypertensive DOCA-salt–treated and control rats. Vasopressin, when administered with currents of 10 to 90 nA, produced a current-dependent increase in firing rate in neurons from both DOCA-treated and control rats. However, at both 10 and 90 nA, prehypertensive DOCA-salt–treated rats showed significantly greater increases in frequency than control rats, as reflected by the greater ratios of response frequency to basal frequency. At 10 nA, control rats showed a 22% increase from basal firing rate, whereas DOCA-salt–treated rats showed a 47% increase. At 90 nA, control rats showed a 68% increase in firing rate, whereas DOCA-salt–treated rats showed a 123% increase.

The increased responsiveness of neurons in DOCA-treated rats was apparent not only in the prehypertensive stage, but also in the chronic hypertensive stage. Figure 2 shows the ratios of response frequency to basal frequency in noradrenergic neurons from rats in the chronic stage of DOCA-salt hypertension and controls in response to microiontophoresis of vasopressin. Neurons from rats with chronic DOCA-salt hypertension showed significantly greater increases in frequency than neurons from control rats in response to all current strengths tested. At 10 nA, control rats showed a 20% increase in firing rate, whereas rats with chronic DOCA-salt hypertension showed a 54% increase. At 30 nA, control rats showed a 40% increase in firing rate, whereas rats with DOCA-salt hypertension showed an 83% increase in firing rate. Finally, at 90 nA, control rats showed an 83% increase in firing rate, whereas DOCA-salt–treated rats showed a 169% increase.

Statistical comparison of DOCA-treated rats and control rats, and rats in the prehypertensive and chronic hypertensive stages showed that neurons from all DOCA-treated rats (regardless of stage) had significantly greater responses to all doses of vasopressin than all control rats (regardless of stage; p<0.0001). The differences in response between DOCA-treated and control rats to all doses of vasopressin were slightly but not significantly greater (p = 0.078) in chronic-stage rats than in prehypertensive rats. The relationship between neuronal responses and dose levels of vasopressin was as follows: 1) the response to vasopressin was dose-dependent (p<0.0001); 2) as the dose of vasopressin increased, the difference in neuronal response between DOCA-treated rats and controls was increased (p<0.0002); and 3) the differences between controls and DOCA-treated rats were greater in the chronic stage, but only for the highest dose of vasopressin (90 nA; p<0.018).

Discussion

Vasopressin–containing fibers and terminals have been identified in the locus ceruleus by immunocytochemical studies. The origin of the majority of these fibers appears to be the nucleus interstitialis striae terminalis, although afferent input from the paraventricular nucleus has also been reported. In addition, perikarya immunoreactive for vasopressin have been identified in the locus ceruleus in rats treated with colchicine. Quantitative measurements of vasopressin by radioimmunoassay in discrete regions of the brain and spinal cord generally support the findings from immunohistochemical studies. High concentrations of vasopressin have been found in the locus ceruleus. Our present findings confirm a previous report demonstrating that vasopressin is able to stimulate
neuronal activity in the locus ceruleus. Our data support the hypothesis that vasopressin may function as a neurotransmitter or neuromodulator, altering the activity of specific neuronal pathways involved in arterial pressure regulation. Furthermore, the findings that neurons from rats with DOCA-salt hypertension showed increased responsiveness to vasopressin suggests a potential central mechanism for this peptide in the pathogenesis of DOCA-salt hypertension.

The increase in responsiveness to vasopressin in rats with DOCA-salt hypertension appears to be attributable to a genuine change in the sensitivity to vasopressin rather than a nonspecific change in the excitability of the cell. In a recent study, it was shown that the sensitivity to glutamate of locus ceruleus neurons from rats with DOCA-salt hypertension did not differ from the sensitivity to glutamate of neurons obtained from control rats. Furthermore, we found that the spontaneous firing rate of noradrenergic neurons from rats with chronic DOCA-salt hypertension is significantly lower than that of neurons from control rats. These results are in keeping with a previous study by Olpe et al.

The present findings raise a question about whether the altered sensitivity to vasopressin of locus ceruleus noradrenergic neurons is linked to the pathogenesis of hypertension in DOCA-salt–treated rats. The following observations are in keeping with this notion. A considerable body of evidence suggests that the locus ceruleus is able to affect the cardiovascular system and that its activation is accompanied by an increase in blood pressure.

Electrical stimulation of the locus ceruleus in rabbits, rats, and cats produces frequency-dependent increases in arterial pressure and heart rate that are primarily mediated by stimulation of the sympathetic nervous system. Compared to control rats, rats with DOCA-salt hypertension showed increased sensitivity and responsiveness to locus ceruleus stimulation, not only in the established stage of hypertension, but also during the prehypertensive stage. Moreover, α-adrenergic receptor blockade with phentolamine produced a significantly greater attenuation in the pressor and heart rate response to locus ceruleus stimulation in rats with DOCA-salt hypertension. It has also been shown that bilateral ablation of the locus ceruleus attenuates the development of DOCA-salt hypertension as well as the increase in arterial pressure and heart rate in response to intracerebroventricular administration of vasopressin. In addition, microinjection of vasopressin into the locus ceruleus of conscious rats produces an increase in arterial pressure and heart rate that is also mediated by activation of the peripheral sympathetic nervous system. Taken together, these results suggest a link between vasopressin, the locus ceruleus, and the sympathetic nervous system in DOCA-salt hypertension.

In summary, noradrenergic neurons from the locus ceruleus of rats with prehypertensive-stage and chronic-stage DOCA-salt hypertension show increased responsiveness to the excitatory agent vasopressin. This interesting observation suggests a potential central mechanism of action for vasopressin. However, the precise role of this peptide and the locus ceruleus in the pathogenesis of DOCA-salt hypertension remains to be defined.

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