Oxytocin Pathways Mediate the Cardiovascular and Behavioral Responses to Substance P in the Rat Brain

Tanja Maier, Wen-Jie Dai, Tamás Csikós, Gustav F Jirkowski, Thomas Unger, Juraj Culman

Abstract—Stimulation of brain periventricular and hypothalamic substance P receptors induces a pressor response and tachycardia associated with mesenteric and renal vasoconstriction and hindlimb vasodilation resembling thus the classical defense reaction. This cardiovascular response is brought about by the activation of the sympathetic system and is accompanied by grooming behavior. To address the role of oxytocinergic pathways in the brain in the mediation of these responses, we investigated the effects of central pretreatment of rats with oxytocin antisense, mixed base, and sense oligodeoxynucleotides on mean arterial pressure, heart rate, and grooming behavior induced by intracerebroventricular injections of substance P (50 pmol). Central pretreatment of conscious rats with the oxytocin antisense oligodeoxynucleotide (intracerebroventricular injections, 8 and 4 hours before administration of substance P) attenuated the mean arterial pressure (by 55%) and heart rate responses (by 58%) as well as grooming behavior induced by the peptide. A complete recovery of all substance P-induced responses was observed 28 hours after antisense oligodeoxynucleotide pretreatment. Intracerebroventricular pretreatment of rats with mixed base and sense oligodeoxynucleotides did not affect the cardiovascular and behavioral responses to substance P. The signal for oxytocin mRNA in the paraventricular nucleus was reduced only in rats pretreated with the antisense oligodeoxynucleotide. These results demonstrate that oxytocin neurons in the paraventricular nucleus, which innervate the cardiovascular centers in the hindbrain and the spinal cord, mediate the increases in blood pressure and heart rate induced by stimulation of substance P receptors in the forebrain. These neurons may also transmit signals, which are generated by substance P in the hypothalamus and are responsible for the sympathetic activation in response to stress (Hypertension. 1998;31[part 2]:480–486.)

Key Words: substance P ■ blood pressure ■ heart rate ■ behavior ■ oxytocin ■ brain

Numerous studies have postulated a link between stress, sympathetic activation, and the pathogenesis of arterial hypertension. The sympathetic outflow and blood pressure are principally controlled by complex interactions between areas localized in the lower brain stem, the hypothalamus, and the intermediolateral column of the spinal cord. A number of neurotransmitters and neuromodulators in the brain participate in the regulation of autonomic functions. Substance P (SP), a natural ligand for NK1 receptors, acts in the forebrain as a potent activator of the sympathoadrenal system. Stimulation of forebrain SP receptors are generated in the hypothalamus. However, the question as to which neurotransmitter systems and neural circuits initiate and mediate these responses has not yet been answered. The paraventricular nucleus (PVN) of the hypothalamus represents an important site for the integration of autonomic, visceral, neuroendocrine, and behavioral responses, including those to stress. A variety of neuroactive substances, including oxytocin and vasopressin, were found in the paraventricular nucleus tract, which projects directly to the intermediolateral column at the thoracolumbar level of the spinal cord. Recent studies have suggested that oxytocin pathways originating in the PVN and innervating the cardiovascular centers in the lower brain stem and sympathetic preganglionic neurons in the spinal cord mediate the tachycardic response to stress. Because the cardiovascular responses induced by stimulation of SP receptors in the forebrain and by stress are virtually identical, we hypothesize that brain oxytocin pathways originating in the PVN may also mediate the cardiovascular response produced by SP.

In recent years, antisense oligonucleotides specifically designed to interact with mRNA coding for peptides or their
receptors have been used increasingly to study physiological functions of various regulatory peptides in freely moving conscious animals. This experimental approach offers the possibility of blocking specific gene expression without multiple, nonspecific side effects.\(^\text{14,15}\) In the present study, experiments were carried out to evaluate the role of oxytocin pathways in the mediation of SP responses by testing the effects of an intracerebroventricular (ICV) pretreatment of rats with an antisense oligodeoxynucleotide directed against the mRNA for oxytocin gene expression on the cardiovascular and behavioral responses induced by SP in the forebrain.

### Materials and Methods

#### Animals and Surgery

Male Wistar rats weighing 300 to 350 g were obtained from Charles River (Sulzfeld, Germany). Rats were housed on a 12/12-hour light/dark cycle with free access to food and water.

#### Surgical Methods

For ICV injections, indwelling polyethylene cannulae (PP 20, LHD, Heidelberg, Germany) were implanted under chloral hydrate anesthesia (400 mg/kg, IP) into the left lateral ventricle 7 to 10 days before the experiment. The stereotactic coordinates were 1.3 mm lateral to the midline, 0.6 mm posterior to the bregma, and 5 mm vertical from the skull surface. Five days after surgery, angiotensin II (25 pmol ICV) was injected. Only those rats that responded by immediate drinking were included in further experiments.

Two days before the experiment, rats were anesthetized again and a polyethylene catheter (PP 50, LHD) filled with heparinized saline was inserted through one femoral artery into the abdominal aorta and then passed through a subcutaneous tunnel, sealed, and secured at the back of the neck. The catheter was used for blood pressure measurements.

#### General Procedures

**ICV Injections of Oligonucleotides and SP**

On the basis of the sequence of oxytocin cDNA, a 22-base 5′-3′-end capped phosphorothioate antisense oligodeoxynucleotide was used that corresponded to the initiation codon of the mRNA. The sequence of the antisense oligomer was 5′-TTTCTCTgTAgTggC-CAgTggA-3′. Rats pretreated with vehicle, the mixed base sequence 5′-gAgggAgAggAAATCTgTTTT-3′, and the sense oligodeoxynucleotide 5′-AggCcTCTgCCCCCAgTCCTg-3′ were used as controls. Oligodeoxynucleotides were dissolved in sterile saline (0.9% NaCl) and injected ICV at a dose 15 μg/15 μl twice (see protocol). Oligodeoxynucleotides (in a volume of 1.5 μl flushed with 5 μl of saline) were slowly infused into the lateral ventricle using a Hamilton syringe connected to a 25-gauge injection needle via polyethylene tubing. Oligodeoxynucleotides were administered slowly over a 40-second period, with the injection needle left in the ICV cannula for 3 to 4 minutes to ensure complete injection of the oligomers. The dose and injection interval were chosen based on results of pilot studies and data published previously.\(^\text{16}\)

**SP** (50 pmol ICV) was injected in a volume of 1 μl flushed with 4 μl of saline.

#### Measurement of Mean Arterial Pressure and Heart Rate

All experiments were performed in conscious rats 48 hours after surgery. Measurements of mean arterial pressure (MAP) and heart rate (HR) after ICV injections of SP or vehicle (saline) were performed using pressure transducers (DTX/Plus, Spectramed Inc., Oxnard, Calif.) connected to a pressure processor (Gould Inc., Valley View, Ohio) coupled to a Gould Brush recorder (Gould Series 2400, Gould Inc.). The analog output signals of MAP and HR from the Gould Brush pressure computer were digitized and then processed using a computer program. This program permits sampling of hemodynamic data from experimental animals directly onto a hard disk of the computer and subsequent analysis with an interactive and graphic program. The hemodynamic data are sampled, compressed, and stored continuously in real time during the entire experiment. The analysis of MAP and HR changes was performed by a computer program as described recently.\(^\text{17}\) Both parameters are expressed as area under the curve (MAP=mm Hg·min, HR=beats·min⁻¹). The value represents the sum of MAP or HR changes integrated in time, i.e., one number quantitatively describes the whole MAP or HR response.

#### Assessment of Behavioral Activity

Behavioral responses to ICV administration of SP were recorded with the animals in cages with grid cage tops removed over a 20-minute period starting immediately after saline or SP injections. The frequency of the behavioral manifestations of face washing/head scratching (FW) and hindquarter grooming/biting (HG) was determined according to the 13-second sampling procedure, as described previously.\(^\text{18}\) Both parameters were expressed as area under the curve. The sections containing the PVN were processed for in situ hybridization as described in detail elsewhere.\(^\text{19}\)

#### In Situ Hybridization

In the present study, a 25-mer oligonucleotide complementary to oxytocin mRNA (5′-CTCggAaAgAaCgACTCgtACg-3′) was used as a probe. The oligonucleotide probe was labeled with digoxigenin using DIG Oligonucleotide Tailing Kit (Boehringer Mannheim, Germany).

Brains were cut in a cryostat into serial frontal sections (50 μm) at −20°C. The sections containing the PVN were processed for in situ hybridization as described in detail elsewhere.\(^\text{19}\)

#### Experimental Protocols

Experiments were conducted 48 hours after the implantation of the femoral catheter. Rats received two ICV injections at 4-hour intervals with vehicle (saline (n=9), antisense (n=10), mixed base (n=9), and sense (n=12) oligodeoxynucleotide (15 μg of each). The first ICV injection of vehicle or oligodeoxynucleotides was given between 8 and 9 AM and the second between 12 AM and 1 PM, exactly 4 hours after the first one. Four hours after the second ICV injection, SP (50 pmol ICV) was injected, and the cardiovascular and behavioral responses were recorded over a 20-minute period. Control rats (n=9) received 5 μl saline without any pretreatment. The ICV injection of saline was used to determine the cardiovascular effects induced by vehicle (saline), the behavioral responses represent the spontaneous behavioral activity over a 20-minute period after vehicle injection. Twenty-four hours after the first SP injection, rats received the second injection of SP (50 pmol ICV) to test the reversibility of any changes in SP responses induced by treatment of rats with oligodeoxynucleotides on the previous day.

A second set of experiments was conducted to evaluate the effect of oligodeoxynucleotide treatment on oxytocin mRNA levels in the PVN. The protocol was similar to that described above. Rats (n=6 per group) underwent ICV pretreatment with vehicle (saline) or oligodeoxynucleotides. Four hours after the second ICV injection, three rats in each group were deeply anesthetized and intracardially perfused with PBS followed by 4% paraformaldehyde solution. The remaining rats were perfused on the next day, 24 hours after the second ICV injection of vehicle or oligodeoxynucleotides. The fixed brains were removed, postfixed overnight in 4% paraformaldehyde, and then incubated for 72 hours in 30% sucrose at 4°C for cryopro-
TABLE 1. Resting Mean Arterial Pressure and Heart Rate Values 24 Hours Before, 4 and 28 Hours after Intracerebroventricular Treatment of Rats With Vehicle, Antisense, Mixed-Base, and Sense Oligodeoxynucleotides

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h Before</td>
<td>4 h After</td>
</tr>
<tr>
<td></td>
<td>24 h Before</td>
<td>4 h After</td>
</tr>
<tr>
<td>Vehicle</td>
<td>93 ± 1</td>
<td>96 ± 2</td>
</tr>
<tr>
<td>Antisense ODN</td>
<td>98 ± 2</td>
<td>105 ± 3*</td>
</tr>
<tr>
<td>Mixed base ODN</td>
<td>97 ± 2</td>
<td>96 ± 3</td>
</tr>
<tr>
<td>Sense ODN</td>
<td>96 ± 3</td>
<td>100 ± 2</td>
</tr>
</tbody>
</table>

Values represent the means ± SEM of (n) rats (vehicle n=9, antisense oligodeoxynucleotide [ODN] n=10, mixed base ODN n=9, sense ODN n=12)

*P< 0.05, statistical comparison to the corresponding value recorded 24 hours before ICV treatment, calculated with ANOVA followed by Duncan test

Statistical Analysis of Data

All values are expressed as means ± SEM Data were subjected to one-way analysis of variance (ANOVA) Duncan test was used as a follow-up test to analyze differences between groups. A significance level of P< 0.05 was accepted.

Results

Pretreatment of rats with the sense oligodeoxynucleotide increased the resting MAP slightly but significantly (Table 1). Fig 1 shows representative profiles of MAP and HR changes induced by ICV injection of SP 4 hours after pretreatment of rats with vehicle or the antisense oligodeoxynucleotide oligomer. Pretreatment of rats with the oxytocin antisense significantly reduced the MAP and HR responses to SP. The effects of ICV pretreatment with vehicle or oligodeoxynucleotides on MAP and HR responses to ICV administration of SP expressed as area under the curve are shown in Fig 2. ICV injection of SP induced marked increases in MAP and HR in the vehicle-treated group. Pretreatment with the antisense oligodeoxynucleotide significantly reduced both responses (MAP by 55%, HR by 58%) to values that did not significantly differ any more from values obtained in controls injected with saline. MAP and HR responses in rats pretreated with mixed base and sense oligodeoxynucleotides did not differ significantly from responses obtained in vehicle-pretreated, SP-injected rats, although the MAP response tended to be reduced somewhat in rats pretreated with the sense oligodeoxynucleotide. The HR responses to SP were slightly attenuated in rats pretreated with both sense and mixed-base oligodeoxynucleotides. Treatment of rats with the antisense oligodeoxynucleotide also attenuated the maximal increases in MAP and HR induced by ICV administration of SP (Table 2).

ICV treatment with oligodeoxynucleotides did not affect the resting MAP and HR values 28 hours afterward. A slight, significant increase in the resting MAP was observed only in rats pretreated with vehicle (Table 1). Pressor and HR responses to ICV injection of SP 24 hours after the first SP injection were similar in rats pretreated with vehicle and oligodeoxynucleotide, indicating that the antisense oligodeoxynucleotide-induced inhibition of the cardiovascular responses to SP was temporary and completely reversible (Table 3).

The cardiovascular response to SP was associated with increased locomotion and intense grooming behavior. FW and HG represent the most prominent behavioral variables ob-

\[ \text{Figure 1. Representative profiles of MAP and HR responses to ICV injection of vehicle (A), substance P (50 pmol) after ICV pretreatment with vehicle (B), and substance P (50 pmol) after ICV pretreatment with the oxytocin antisense oligodeoxynucleotide (C). The time point of the ICV injection of vehicle or substance P is indicated by the arrow.} \]
served after stimulation of forebrain SP receptors. Pretreatment of rats with the antisense oligodeoxynucleotide effectively inhibited SP-induced FW compared with rats pretreated with vehicle, mixed-base, and sense oligodeoxynucleotide (Table 4). SP-induced HG behavior was also attenuated in the group pretreated with antisense oligodeoxynucleotide when compared with rats pretreated with vehicle or sense oligodeoxynucleotide. Because SP-induced HG was somewhat less pronounced in rats pretreated with the mixed-base oligodeoxynucleotide, the difference between the values in the rats pretreated with antisense and the group of rats pretreated with mixed base oligodeoxynucleotide was not statistically significant. The oxytocin antisense oligodeoxynucleotide-induced inhibition of FW and HG was not observed when SP was injected 24 hours after the last antisense oligodeoxynucleotide injection (data not shown).

### Table 2. Maximal Increases in Mean Arterial Pressure and Heart Rate Induced by Substance P (50 pmol) Injected Intracerebroventricularly 4 Hours After Pretreatment With Vehicle, Antisense, Mixed-Base, and Sense Oligodeoxynucleotides

<table>
<thead>
<tr>
<th>Group (ICV pretreatment)</th>
<th>Injection</th>
<th>(\Delta)MAP (mm Hg)</th>
<th>(\Delta)HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>saline</td>
<td>24±2</td>
<td>131±10</td>
</tr>
<tr>
<td>Antisense ODN</td>
<td>saline</td>
<td>14±3*</td>
<td>70±14*</td>
</tr>
<tr>
<td>Mixed base ODN</td>
<td>saline</td>
<td>25±1</td>
<td>139±11</td>
</tr>
<tr>
<td>Sense ODN</td>
<td>saline</td>
<td>24±1</td>
<td>133±8</td>
</tr>
</tbody>
</table>

* Values represent the means±SEM of (n) rats.
* Statistical comparison was calculated with ANOVA followed by Duncan test.

### Table 3. Mean Arterial Pressure and Heart Rate Responses Induced by Substance P (50 pmol) Injected Intracerebroventricularly 28 Hours After Pretreatment With Vehicle, Antisense, Mixed-Base and Sense Oligodeoxynucleotides

<table>
<thead>
<tr>
<th>Group (ICV injection)</th>
<th>Injection</th>
<th>MAP (AUC) (mm Hg×min)</th>
<th>HR (AUC) (beats/min×min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>saline</td>
<td>17±7</td>
<td>83±32</td>
</tr>
<tr>
<td>Vehicle SP</td>
<td>SP</td>
<td>105±14</td>
<td>768±69</td>
</tr>
<tr>
<td>Antisense ODN SP</td>
<td>SP</td>
<td>119±15</td>
<td>553±68</td>
</tr>
<tr>
<td>Mixed base ODN SP</td>
<td>SP</td>
<td>104±11</td>
<td>500±87</td>
</tr>
<tr>
<td>Sense ODN SP</td>
<td>SP</td>
<td>88±16</td>
<td>682±97</td>
</tr>
</tbody>
</table>

* Values represent the means±SEM of (n) rats. Mean arterial pressure (MAP) and heart rate (HR) are expressed as area under the curve (AUC) (MAP-mm Hg×min, HR-beats/min×min). AUC was calculated for a period of 20 minutes starting at the time of the intracerebroventricular (ICV) injection. The values in vehicle and oligodeoxynucleotide (ODN)-treated groups did not differ significantly.

**Discussion**

The considerable potency of SP in the forebrain to activate the sympatho-adrenal system points to a role of this peptide in eliciting cardiovascular response to stress. Indeed, we have recently demonstrated in rats that an inhibition of central NK1 receptors attenuates the pressor, HR, and behavioral responses to noxious stimuli. The hypothalamus, especially the PVN, has been proposed as a site of the generation of these responses. In the PVN, the mRNA signal for oxytocin is present in cells that are more frequent, outnumbering AVP-stained neurons by a ratio of 3 to 1. It seems that oxytocin neurons in the PVN can substantially contribute to control of sympatho-adrenal activity through direct action on sympathetic preganglionic neurons.

Recently, antisense oligodeoxynucleotides have been used in vivo experiments to study the physiological functions of neuropeptides and their receptors in the brain. Here, we demonstrate that specific targeting of oxytocin neurons with the antisense oligodeoxynucleotide directed against the mRNA for oxytocin reduced the pressor response and tachycardia as well as the behavioral response induced by central injection of SP. The effect of the antisense oligodeoxynucleotide treatment on these responses was only transient,
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TABLE 4. Face Washing/Head Scratching (FW) and Hindquarter Grooming/Biting (HG) Induced by Substance P (50 pmol) Injected Intracerebroventricularly 4 Hours After Pretreatment With Vehicle, Antisense, Mixed Base, and Sense Oligodeoxynucleotides

<table>
<thead>
<tr>
<th>Group (ICV pretreatment)</th>
<th>ICV Injection</th>
<th>n</th>
<th>FW</th>
<th>HG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls Saline</td>
<td>9</td>
<td>1.2±0.7</td>
<td>1.5±0.5</td>
<td></td>
</tr>
<tr>
<td>Vehicle SP</td>
<td>9</td>
<td>8.1±1.7</td>
<td>7.6±1.3</td>
<td></td>
</tr>
<tr>
<td>Antisense ODN SP</td>
<td>10</td>
<td>2.7±1.0*†‡</td>
<td>1.4±0.7*‡</td>
<td></td>
</tr>
<tr>
<td>Mixed base ODN SP</td>
<td>9</td>
<td>7.0±1.3</td>
<td>5.1±1.9</td>
<td></td>
</tr>
<tr>
<td>Sense ODN SP</td>
<td>12</td>
<td>7.3±1.4</td>
<td>6.5±1.6</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent the frequency of individual behavioral manifestations for 20 minutes and are indicated by the means±SEM of (n) rats. The values in all groups except for the antisense oligodeoxynucleotide (ODN)-treated group differ significantly from controls (significance not shown).
†P<.05 vs vehicle-treated group.
‡P<.05 vs mixed-base ODN-treated group.
$P<.05 vs sense ODN-treated group; statistical comparisons calculated with ANOVA followed by Duncan test.

because the inhibition was no longer observed when SP was injected 28 hours after the nucleotide treatment.

In most of the in vivo studies, antisense oligodeoxynucleotides were administered using multiple injections or infusions over several days, but very rapid effects after a single antisense oligodeoxynucleotide injection could also be demonstrated. The efficiency of the two consecutive ICV injections of the antisense probe used in the present study to affect the function of oxytocin neurons in the PVN was controlled by an in situ hybridization analysis for the oxytocin mRNA signal in the PVN. It is assumed that after crossing the cell membrane, antisense oligonucleotides hybridize to the target mRNAs, forming a substrate for nucleases. Correspondingly, in the present study, ICV treatment of rats with the antisense oligodeoxynucleotide reduced the oxytocin mRNA signal in the PVN, as revealed by in situ hybridization analysis. These findings indicate that the dose of the oxytocin antisense oligodeoxynucleotide was sufficient to temporarily alter the production of oxytocin, which might be associated with modification of neuronal activity and excitability.

The antisense probe used in the present study contained a G-tetrad. Although antisense experiments in vitro have shown that probes containing repeated G-sequences also induce toxic effects, eg, by blocking the DNA-polymerase II, such effects have not been observed in vivo so far. The oxytocin antisense probe used in the present study is unlikely to have exerted toxic actions because of the time course and complete reversibility of the effects of the antisense treatment on the SP-induced cardiovascular and behavioral responses. Oxytocin antisense probes to various different sequences of the oxytocin start coding region, with or without repeated G-sequences, were used in previous studies. None of these probes showed any sequence-independent effects.

The finding of the reduced pressor and HR responses to SP in rats pretreated with the antisense oligodeoxynucleotide indicates that oxytocin neurons in the PVN, which project to the spinal cord, participate in the activation of the sympathoadrenal system in response to stimulation of forebrain NK1 receptors. We assume that the SP-induced activation of neuronal circuits in the hypothalamus stimulates oxytocin neurons in the PVN, which results in an increased release of oxytocin from nerve terminals localized on sympathetic preganglionic neurons in the intermediolateral column of the spinal cord. In general, oxytocin has been reported to excite spinal sympathetic neurons. In the rat, intrathecally administered oxytocin at the thoracic level increased HR, and oxytocin injected at the lumbar level induced a rise in blood pressure.

The mechanisms by which the antisense nucleotide reduces the neuronal activity of oxytocin neurons in the PVN and, consequently, the release of oxytocin from nerve terminals are not known. One of the proposed mechanisms of the antisense oligodeoxynucleotide actions is the reduction of oxytocin synthesis in the neuronal perikarya. Oxytocin synthesized in the PVN is transported to the thoracic spinal cord via a fast component of axonal transport and may thus reach oxytocin terminals localized in the upper thoracic spinal cord within 6 hours. Because 8 hours elapsed between the first antisense probe application and the recording of the cardiovascular

Figure 3. Effects of ICV treatment with vehicle (A), antisense (B), mixed-base (C), and sense oligodeoxynucleotides (D) on oxytocin mRNA hybridization signal in the PVN. Rats were killed 4 hours after the second ICV injection of vehicle or the respective oligodeoxynucleotide.
responses to SP, a rapid inhibition of oxytocin synthesis in the PVN and reduced amounts of the peptide being transported to the spinal cord might decrease the oxytocin content and consequently the release of the peptide from nerve terminals. This, however, may not be true, because the large storage capacity of nerve terminals for oxytocin provides for an adequate release of the peptide when its synthesis is altered. Morris et al.\textsuperscript{11} recently reported a rapid increase in brain stem oxytocin content in response to antisense oligomer infusion into the PVN. It has been hypothesized that the increased levels of oxytocin in the brain stem, which represents a site of the termination of oxytocin fibers deriving from the PVN,\textsuperscript{16} may be related to a reduction in oxytocin release, which would result in an accumulation of the peptide.\textsuperscript{17} The idea is indirectly supported by recent data demonstrating a reduced neuronal excitability of oxytocin neurons after antisense oligodeoxynucleotide treatment.\textsuperscript{19} In view of these findings, we suppose that central treatment with the antisense nucleotide reduces the excitability of oxytocin neurons projecting into the spinal cord and also the release of oxytocin from the nerve terminals localized on sympathetic preganglionic neurons.

Moms et al.\textsuperscript{11} recently demonstrated that infusion of antisense oligodeoxynucleotide in the PVN prevents the tachycardia but not the pressor response induced by shaker stress. Centrally administered vasopressin-oxytocin antagonists or lesions of the PVN also prevented increases in HR induced by stress.\textsuperscript{12} Several lines of evidence indicate that SP belongs to the neuroactive substances in the brain which generate stress-induced pressor responses and tachycardia.\textsuperscript{8} It seems that SP and other neurotransmitters or neuromodulators in the forebrain, most probably in hypothalamic neural circuits, activate oxytocin neurons in the PVN that increase the sympathetic drive, resulting in tachycardia. Although our present data demonstrate that central oxytocin pathways are crucial in mediating the SP-induced pressor responses, neurotransmitter systems other than oxytocin probably mediate increases in blood pressure in response to stress.

It has been reported that oxytocin antisense oligomer injected into the PVN did not affect basal levels of oxytocin in plasma or resting cardiovascular parameters.\textsuperscript{13} In contrast to these findings, we observed that treatment of rats with the oxytocin antisense oligomer slightly increased the resting MAP. Oxytocin fibers deriving from the PVN densely innervate the nucleus tractus solitarii and the dorsal motor nucleus of the vagus nerve,\textsuperscript{10} which represent the key areas in the maintenance of normal and reflex control of blood pressure.\textsuperscript{8} It is, therefore, conceivable to assume that the increased resting MAP might result from a modified release of oxytocin in these areas as a consequence of altered neuronal excitability of oxytocin neurons after antisense oligodeoxynucleotide treatment.\textsuperscript{11}

Treatment of rats with the antisense probe attenuated grooming behavior in response to SP. Excessive grooming and skin biting are the most characteristic behavioral manifestations elicited by stimulation of central NK, receptors.\textsuperscript{8} This behavioral response pattern represents an integral part of the reaction of rodents to noxious stimuli and stress.\textsuperscript{20} We recently reported that the behavioral response to a noxious stimulus can be prevented by inhibition of central NK1 receptors.\textsuperscript{17} It seems likely that SP acting on NK1 receptors, located most probably in the hypothalamic neural circuitry, activates oxytocin neurons in the PVN which then initiate and mediate the behavioral effects. Although oxytocin administered ICV has been reported to induce an intense grooming behavior,\textsuperscript{21} it remains to be established in which brain areas the release of oxytocin evokes grooming behavior in the rat.

Our current studies indicate that central oxytocin pathways projecting to the hindbrain and the spinal cord mediate SP-induced sympathoadrenal activation. In view of previous findings demonstrating that SP in the brain represents a potent activator of the sympathoadrenal system and participates in the generation of the cardiovascular responses to stress, the present data contribute to our understanding of the mechanisms leading to the sympathoadrenal inhibition observed after lesions of the PVN and may also help to explain why lesions of this area prevent the development of spontaneous hypertension.\textsuperscript{28}

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