Effect of the Substance P Antagonist Spantide on Adrenal Sympathetic Nerve Activity in Rats

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Abstract—In order to elucidate the role of substance P in adrenal nerve activity, the effects of substance P antagonists on 1) tonic activity of the adrenal sympathetic nerve, 2) the response to exogenous substance P and 3) cutaneo-adrenal nerve reflex were investigated in anesthetized rats. Substance P or its antagonist [(Arg', D-Trp7'9, Leu'')-substance P: spantide] was injected into the subarachnoid space via polyethylene tubing. Intrathecal administration of spantide (0.1–10 μg) produced a slight and transient increase in tonic activity of the adrenal nerve. Thereafter, a marked and prolonged decrease in adrenal nerve activity was observed, along with a decrease in arterial blood pressure. Intrathecally administered substance P (0.1–10 μg) produced a marked increase in tonic activity of the adrenal nerve in a dose-related manner. This substance P-induced increase in adrenal nerve activity was significantly antagonized by pretreatment with spantide. It was also found that spantide depressed the reflex response in adrenal nerve activity evoked by noxious mechanical pinching of the skin. These findings suggest the possibility that on the spinal level, substance P has an important role in the maintenance of tonic activity of the adrenal nerve and the transmission of nociceptive information to the adrenal nerve in rats.

The undecapeptide substance P is present in numerous intrinsic neural pathways throughout the central nervous system (1–4) and the peripheral nervous tissues (5, 6). Much evidence has accumulated supporting the suggestion that substance P has an excitatory role in neural transmission involving spinal nociceptive pathways (7–12). Recently, this peptide has gained interest as a blood pressure regulating neuropeptide because of its localization in brain areas involved in cardiovascular control such as the medulla oblongata and the hypothalamus (13–15). Furthermore, it has been reported that substance P neurons originating in the ventral medulla oblongata project to the intermediolateral cell column in the spinal cord (16), from which the preganglionic sympathetic nerve originates (17). This evidence raises the possibility that substance P may be involved in the control of sympathetic nerve activity.

(D-Arg', D-Trp7'9, Leu'')-substance P belongs to a series of full length substance P analogues with amino acid substitution. This compound, which was named as spantide by Folkers, blocked the contractile effect of substance P on isolated guinea pig taenia coli in a competitive manner with little or no agonistic action and histamine releasing action (18), and it antagonized substance P binding in the central nervous system (19). Using this antagonist, we undertook the present experiments to elucidate the role of substance P in adrenal sympathetic nerve activity in anesthetized rats. In this study, the
following three aspects of the effects of span tide were studied: 1) effect on tonic activity of the adrenal sympathetic nerve, 2) antagonism to exogenous substance P-induced response of adrenal nerve activity, and 3) effect on adrenal nerve activity evoked by noxious mechanical stimulation of the skin (cutaneo–anrenal nerve reflex).

Materials and Methods

Animals: Adult male Wistar rats, weighing 360 to 470 g, were anesthetized with α-chloralose and urethane (50 mg/kg and 500 mg/kg, i.p., respectively) with a supplemental dose given intravenously as needed. After immobilization with gallamine trichloride (10–20 mg/kg, i.v., Sigma Chemical Company), respiration was maintained with a tracheal cannula which was connected to an artificial respirator (model 131, Princeton Medical Instruments, Inc., Natick, MA). Rectal temperature was maintained between 37°C and 38°C with a DC current heating pad. Blood pressure and heart rate were monitored continuously via the right femoral artery.

Drugs: Substance P (Peptide Institute, Inc.) and (D-Arg¹, D-Trp⁷,⁹, Leu¹¹)-substance P (span tide, synthetized by M. Fujino and C. Kitada) were dissolved in artificial cerebrospinal fluid containing bovine serum albumin (20) to give a stock solution of 2 mg/ml. This solution was divided in small samples which were kept frozen until used. Further dilutions were made with 0.9% saline before using. The test solutions were injected into the subarachnoid space via polyethylene tubing, which was inserted through an incision in the atlantocipital membrane to the caudal region of the thoracic enlargement (T 10). The injection volume was 5 μl, flushed with 10 μl of saline. Correct placement of the cannula in the spinal cord was verified by an intrathecal injection of black dye after the animal had been sacrificed.

Adrenal nerve recordings: The left adrenal branches from the splanchnic nerve were dissected retroperitoneally and cut near the adrenal gland. Efferent nerve mass discharges were recorded from the central cut end of the nerve with bipolar platinum-iridium wire electrodes. Adrenal nerve activity was amplified, passed through a window discriminator in order to pick out discharges from background noise, and counted every 10 sec (Channel Selector S-1526, Nihon Kohden) (21).

Cutaneous stimulation: Noxious mechanical stimulation was performed by pinching skin areas of approximately 1 cm² with a surgical clamp (about 5 kg force). Pinching was applied to different cutaneous segmental areas: the lower chest from whose spinal segments the adrenal nerves emerge (22) and the hindpaw whose spinal segments are distal to the segments of the adrenal sympathetic outflow (23). These cutaneous areas were stimulated for 1 min, before (control period) and 10 to 30 min after span tide administration, respectively. In some cases, pinching stimulation was also applied after adrenal nerve activity had recovered. Reflex response of adrenal nerve activity to the mechanical stimulation was expressed as the percentage of change in the number of nerve impulses.

Calculation and the statistical analysis: The data are expressed as the mean±S.D. Statistical differences between two means (P less than 0.05) were determined by Student’s t-test. When two or more treatment means were compared with one mean, differences between group means were determined with an analysis of variance. The critical differences of group means were assessed with the modified t-test according to Bonferroni (24).

Results

Effect of exogenous substance P on tonic activity of the adrenal sympathetic nerve: As shown in Fig. 1, intrathecal administration of synthetic substance P at a dose of 0.1 to 10 μg produced an immediate and marked increase in adrenal nerve activity. The magnitude of these excitatory responses and the delays in return were dose-related. The mean response induced by 10 μg substance P was significantly different from that of the vehicle-administered control up to 4 min after injection. Intrathecal administration of substance P did not produce any significant change in arterial blood pressure or heart rate. Infusion of vehicle into the subarachnoid
Fig. 1. Effect of intrathecal substance P on spontaneous adrenal efferent nerve activity in rats. (A): time course observation. Each point represents the mean±S.D. The ordinate is the percentage of change in the adrenal nerve activity from the value obtained before substance P administration as indicated by the arrow (□-----□, 0.1 µg, n=4; □-----□, 1 µg, n=6; □—□, 10 µg, n=6; △—△, vehicle, n=6). The abscissa is the time in minutes after administration. *P<0.05, **P<0.01 and ***P<0.001 vs. vehicle-administered control. (B): representative recordings of adrenal nerve activity and simultaneously measured blood pressure and heart rate, obtained from the rats that were given 1 µg substance P.

space had no effect on adrenal nerve activity, blood pressure and heart rate (Table 1).

Effect of spantide on tonic activity of the adrenal sympathetic nerve: Intrathecal administration of the substance P antagonist spantide initially caused a slight and transient increase and then a marked and prolonged decrease in tonic activity of the adrenal nerve. This spantide-induced decrease in adrenal nerve activity recovered with the lapse of time. Specimen recording and observations of adrenal nerve activity over a period of 60 min are shown in Fig. 2. Maximum decrease in adrenal nerve activity was dose dependent and was significantly different from that in the vehicle injected group (Fig. 3). Although simultaneously measured blood pressure and heart rate also changed with the nerve activity, some difference in time course and effective doses were observed; a significant change was observed only in the mean blood pressure of the group which received 10 µg of spantide (Table 1).

Antagonism of spantide on exogenous substance P: The effect of intrathecally administered spantide on the exogenous substance P-induced response in adrenal nerve activity was examined. Ten to 15 min after pretreatment of 10 µg of spantide or vehicle, 0.1, 1 or 10 µg of substance P was administered intrathecally. The increase in adrenal nerve activity, which was expressed as the percent change of the activity counts before substance P injection, was compared with that of the corresponding controls: vehicle-administered controls with pre-treatment of spantide or vehicle.

As shown in Fig. 4, intrathecal injection of substance P (0.1–10 µg) caused dose-dependent increases in the maximal responses of adrenal nerve activity. This substance P-induced excitation in the adrenal nerve was significantly antagonized by pretreatment with 10 µg of spantide.

Effect of spantide on adrenal nerve activity evoked by noxious mechanical stimulation of the skin: Reflex responses in adrenal nerve activity elicited by cutaneous pinching were compared before and after intrathecal injection of spantide at a dose of 10 µg. Specimen recordings obtained from one rat are depicted in Fig. 5. In this case, lower chest pinching was unable to produce a
Table 1. Effects of intrathecal administration of substance P and its antagonist on mean arterial blood pressure and heart rate in anesthetized rats

<table>
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<th>before administration</th>
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Values indicate the mean±S.D. *P<0.05 vs. vehicle-administered control.
Fig. 2. Effect of intrathecal spantide on spontaneous adrenal efferent nerve activity in rats. (A): representative recording of adrenal nerve activity and simultaneously measured blood pressure and heart rate, obtained from a rat that was given 10 μg spantide. (B): time course observation. Each point represents the mean±S.D. The ordinate is the percentage of change in adrenal nerve activity from the value obtained before spantide administration as indicated by the arrow (●), 0.1 μg, n=4; ○, 0.1 μg, n=5; ■, 10 μg, n=8; △, vehicle, n=6). The abscissa is time in minutes. *P<0.05, **P<0.01 and ***P<0.001 vs. vehicle-administered control.

Discussion

The present study demonstrated that substance P antagonist, spantide [(D-Arg¹,D-Try⁷⁻⁹, Leu¹¹)-substance P], depressed tonic activity of the adrenal nerve in anesthetized rats. Blood pressure also decreased after spantide administration. Moreover, spantide antagonized the excitatory responses induced by exogenous intrathecal substance P.

It has been reported that fibers originating in the ventral medulla oblongata innervate the intermediolateral cell column of the spinal cord (25-27), and electrical lesions of the ventral medulla oblongata including the nucleus interfascicularis hypoglossi produces a decrease in the substance P-like immunoreactivity in the intermediolateral cell column (16). Furthermore, Takano et al. (28) reported that the stimulation of the ventral medulla oblongata by the excitotoxic agent, kainic acid, elicited a release of immunoreactive substance P in the spinal cord superfusion samples. This release of substance P correlated with the rise in blood pressure. These facts suggest that the substance P-like neurons of the ventral medulla may play a role in maintaining vasomotor tone. Concerning tonic activity of the adrenal nerve, Araki et al. (23) suggested that some excitatory control from the supraspinal structures to the adrenal medulla exists in animals with an intact central nervous system, since adrenal sympathetic nerve
activity and catecholamine secretion from the adrenal medulla were markedly reduced by acute spinal transection, and these were accompanied with a decrease in arterial blood pressure. Accordingly, our finding that spantide decreased adrenal nerve activity suggests that intrinsic substance P may have a critical role in maintenance of the tonic activity of the adrenal nerve.

The reason for the slight increase in adrenal nerve activity as well as in blood pressure for several minutes after spantide injection was not clarified in the present study. However, it possibly may be due to an interaction with other peptides on the superficial laminae of the spinal cord. Furthermore, it is possible that the agonistic action of spantide may be related to the spantide-induced initial increase in adrenal nerve activity.

The present study also demonstrated that spantide depressed the reflex response in adrenal nerve activity which was elicited by noxious mechanical stimulation of the skin. Yaksh et al. (29) demonstrated that the activation of nociceptive sensory afferents releases substance P-like immunoreactivity from the cat spinal cord. This evidence leads us to suggest that substance P may be involved in the transmission of noxious peripheral stimuli to the adrenal nerve and that the spantide-induced decrease in cutaneo-adrenal nerve reflex may be due to its antagonistic action to the intrinsic substance P.

It has been reported that the reflex response elicited by lower chest pinching is mediated via both supraspinal and spinal structures and the reflex response elicited by hindpaw pinching is mainly mediated via the supraspi-
Fig. 5. Representative recordings illustrating the effect of intrathecal spantide on the evoked adrenal nerve activity by noxious lower chest pinching in the rat. (A)-(C): recordings of adrenal nerve activity and simultaneously measured blood pressure and heart rate, obtained from one rat. The lower chest was pinched for 1 min as indicated by bars, before (A), 15 min (B) and 90 min (C) after 10 \( \mu \)g spantide, respectively.

Fig. 6. Effect of intrathecal spantide on adrenal nerve activity evoked by noxious cutaneous pinching in rats. Cutaneous stimulation was applied to the lower chest (upper panel) and hindpaw (lower panel) for 1 min, before and 10 to 30 min after intrathecal spantide as indicated by the hatched bar. Each value indicates the mean±S.D. of the increase in adrenal nerve activity during pinching, which is expressed as the percentage of change from the value obtained before stimulation. The number of animals is given in parentheses. *P<0.05 and **P<0.01 vs. the value before spantide.

It has also been reported that spantide antagonized a bombesin-like peptide binding in the central nervous system (19), and other tachykinin families such as neurokinin A or B exist in the rat spinal cord (35).

In summary, the present study demonstrated that spantide depressed the tonic activity of the adrenal nerve. Exogenous substance P, which was administered intrathecally, caused an excitatory response in adrenal nerve activity, and this response was blocked by the pretreatment with intrathecal spantide. These results indicate the possibility that the spantide-induced decrease in tonic activity of the adrenal nerve is due to the blocking action of endogenous substance P. It was also found that spantide depressed the reflex in adrenal nerve activity evoked by noxious cutaneous pinching. These findings suggest that on the spinal level, substance P has a role not only in maintenance of the tonic activity of the adrenal nerve, but also in the transmission of nociceptive information.
to the adrenal nerve in rats.

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