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Cardiovascular effects of substance P and capsaicin microinjected into the nucleus tractus solitarii of the rat

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This report deals with the effect of substance P (SP) and capsaicin on blood pressure and heart rate after administration into different sites of the nucleus tractus solitarii (NTS) of urethane-anesthetized rats. Microinjection of SP at 6 different coordinates throughout the NTS showed 3 sites where SP administration evoked changes in blood pressure and heart rate. The most sensitive sites where application of SP into the NTS evoked dose-dependent hypotension and bradycardia were at the level of the posterior tip of the area postrema (zero level) and at the level of the obex. Capsaicin evoked dose-dependent hypotension and bradycardia at the same sites. These results further support the possibility that SP may be a neurotransmitter or neuromodulator of baroreceptor afferents in the NTS.

INTRODUCTION

Substance P (SP), a member of the tachykinin family, was initially isolated from bovine hypothalamus[3]. A high concentration of SP has been detected in discrete brain areas[25,33]. A high content of SP and the distribution of SP-immunoreactive nerve terminals in the nucleus tractus solitarii (NTS) has been reported[20,33]. In recent studies[13,17] evidence has been presented that SP occurs in baro- and chemoreceptor afferents which terminate in the NTS. Furthermore, there is evidence[21,28,36] for synaptic connections between catecholaminergic neurons of the A2 group, predominantly located in the caudal part of the NTS and SP-immunoreactive axons. The catecholaminergic neurons of the A2 group are considered to be important for the regulation of blood pressure[5,6], and they may be influenced by SP afferents via synaptic contacts[21]. In the light of the neuroanatomical localization of SP, attempts were made to elucidate the role of SP in the central regulation of the cardiovascular system. Intracerebroventricularly (i.c.v.) or intracisternally (i.c.) administered SP in conscious and in anesthetized rats and rabbits evokes a pressor response[15,26,27,38–40] which seems to be mediated by the sympathetic nervous system[40]. I.c.v. administration of SP causes tachycardia in rats[15,39,40], and bradycardia in rabbits[26,27].

Data regarding the effect of local application of SP into the NTS on blood pressure and heart rate are rather conflicting. Some investigators[2,37] observed that SP does not affect blood pressure or heart rate, while others observed an increase or decrease in blood pressure, heart rate and spontaneous sympathetic nervous activity[14,15].

Capsaicin is an agent known to release substance P from primary afferent fibers[11]. Its cardiovascular effects have been studied extensively after peripheral[8] or central[15,19,30] administration. Administration of capsaicin into the 4th ventricle or cisterna magna results in increases in blood pressure and heart rate[30]. Capsaicin applied to a ventral medullary site[19,30] increases blood pressure[19,30], sympathetic nerve activity[19], and heart rate[30]. Microinjection of this agent

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into the NTS was found to produce a decrease in blood pressure, heart rate and sympathetic nerve activity[15], or no response[30].

The aim of the present study was to determine the role of SP and capsaicin in the NTS in cardiovascular regulation by microinjection of these compounds into different sites of the NTS.

MATERIALS AND METHODS

Male rats of an inbred Wistar strain, weighing 200–250 g were used. The rats were anesthetized with urethane (1.3 g/kg, i.p.). Blood pressure was recorded from an indwelling cannula in the femoral artery using a Statham transducer (Model P23-AC) connected to a Grass polygraph. Each rat was placed in a stereotactic apparatus with the head flexed to 45°. The dorsal surface of the lower brainstem was exposed by limited occipital craniotomy. The caudal tip of the area postrema in the midline was used as the rostrocaudal zero. Microinjections into the area of the NTS were given unilaterally via a glass cannula (outer diameter 60 μm) or a double-barrel cannula (outer diameter 80–100 μm) in a volume of 0.2 μl. This volume was delivered over a period of 10 s. The glass micropipettes and microsyringes were connected by plastic tubing. The glass cannula was filled with SP freshly dissolved in saline (0.9%) or in artificial cerebrospinal fluid (ACSF)[1], or vehicle. Solution of capsaicin was prepared with the aid of 5% ethanol and 5% Tween 80, and appropriate dilutions were made with saline. One minute after each injection the glass cannula was removed from the brainstem and its patency tested by observing the flow during a test trial. After termination of the experiment the rats were killed by decapitation. The brain was removed from the skull and fixed in 10% formalin. Frozen sections of 60 μm were cut and the localizations of the needle-tracks were observed microscopically. SP was obtained from Bachem, Bubendorf, Switzerland, capsaicin from Merck, Darmstadt, F.R.G. and the SP antagonist (D-Pro^2, D-Trp^{7,9})-SP was a gift from Dr. J.M. Stewart, Denver, U.S.A.

All values used in the analysis represent the mean ± S.D. The significance of differences between test animals and their controls was calculated by means of Student's *t*-test for unpaired and paired (experiments with antagonist) data.

RESULTS

Blood pressure and heart rate effects of SP after microinjection into the NTS

Unilateral microinjection of 22.2 pmol SP into the NTS at 5 different sites throughout the NTS resulted in cardiovascular changes or had no effect. Microinjection of 0.2 μl saline into the same sites had no effect on blood pressure and heart rate. SP (22.2 pmol) did not alter blood pressure and heart rate at the coordinates AP –1.0, L 0.2, V 0.7; AP –0.5, L 0.2, V 0.7; AP 0.8, L 0.6, V 0.6 mm. Fig. 1 summarizes the effects of SP (22.2 pmol and 74.0 pmol) on blood pressure and heart rate at the coordinates AP 0.0, L 0.5, V 0.9 (zero level) and AP 1.2, L 0.8, V 0.6 (obex level). SP evoked dose-dependent hypotension and bradycardia after microinjection at the level of the obex (Fig. 1A,C). Microinjection of 22.2 pmol SP resulted in a significant decrease in blood pressure (Fig. 1A) in the first and second minute. The bradycardiac effect (Fig. 1C) was longer lasting. The higher dose of SP evoked significant hypotension (Fig. 1A) and bradycardia (Fig. 1C) immediately after administration. In contrast to the effect of SP at the level of the obex (Fig. 1A,C), the application of the peptide at zero level (Fig. 1B,D) resulted in short-lasting changes in blood pressure and heart rate. These effects of SP at zero level were dose-dependent. Microinjection of SP (22.2 pmol) into tissue surrounding the NTS affected neither blood pressure nor heart rate. SP, in doses from 7.4 fmol to 0.74 pmol, failed to evoke changes in blood pressure and heart rate at zero level.

In a restricted area (coordinates AP +0.42 ± 0.18, L 0.5, V 0.9) the NTS was found to be very sensitive to saline. Saline in a volume of 0.2 μl evoked short-lasting hypotension and bradycardia (△ mean blood pressure –38.6 ± 15.6 mm Hg and △ mean heart rate –37.1 ± 22.0 beats per minute – unpublished results). In contrast to saline, ACSF microinjected at the same site did not induce changes in these parameters (Fig. 2). SP in doses of 74.0 pmol and 222.9 pmol dissolved in ACSF resulted in a dose-dependent increase in heart rate, while blood pressure was not affected.

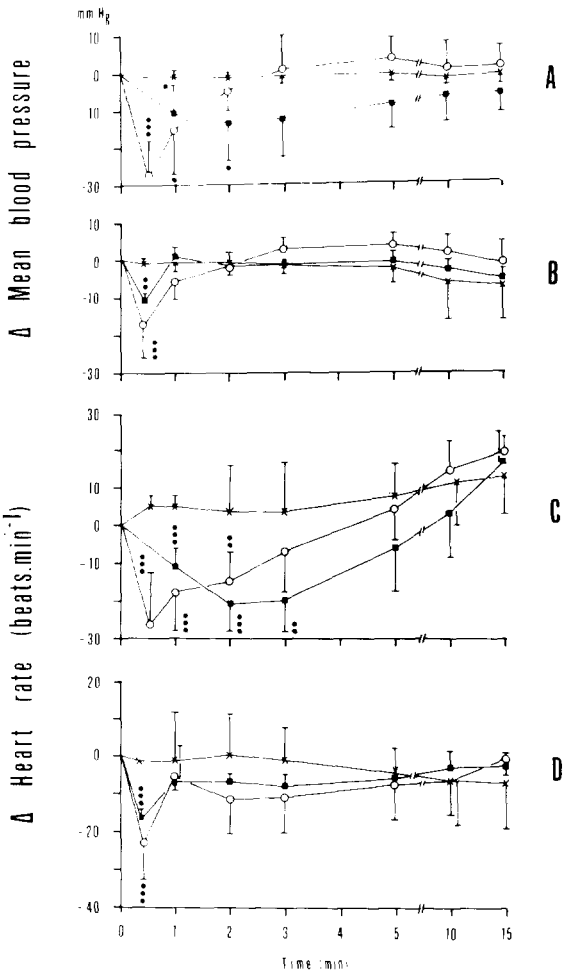

Fig. 1. Effect of substance P (22.2 pmol ■——■, 74.0 pmol ○——○) and saline (×——×) on mean arterial pressure and heart rate after administration into the NTS of urethane anesthetized rats. Substance P and saline were microinjected into the NTS at coordinates AP 0.0, L 0.5, V 0.9 (B,D) and AP +1.2, L 0.8, V 0.6 (A,C). Mean ± S.D.; $n = 6$–8; •: $P < 0.05$; ••: $P < 0.01$; •••: $P < 0.001$.

Effect of SP antagonist (D-Pro², D-Trp⁷,⁹)-SP on cardiovascular effects of SP

In 6 rats two successive microinjections (time interval 20 min) of the same dose of SP (22.2 pmol) were given into the NTS at zero level without changing the position of the cannula. The resulting initial changes in blood pressure and heart rate between the first microinjection of SP (△ mean blood pressure −15.2 ± 10.1 mm Hg and △ mean heart rate −18.2 ± 5.9 beats/min) and the second one (△ mean blood pressure −14.6 ± 5.6 mm Hg and △ mean heart rate −16.0 ± 6.2 beats/min), were not significantly different. By using the double-barrel cannula in a further 6 animals, the SP antagonist (49.4 pmol) was applied 3 min before SP (22.2 pmol) and at the same site. The antagonist did not significantly change the hypotensive and bradycardiac effect of the second application of SP (△ mean blood pressure −17.5 ± 9.2 mm Hg and △ mean heart rate −15.3 ± 7.5 beats/min) in comparison to the first (control) application of SP (△ mean blood pressure −15.5 ± 5.2 mm Hg and △ mean heart rate −16.8 ± 6.4 beats/min). The antagonist alone had no effect on blood pressure and heart rate.

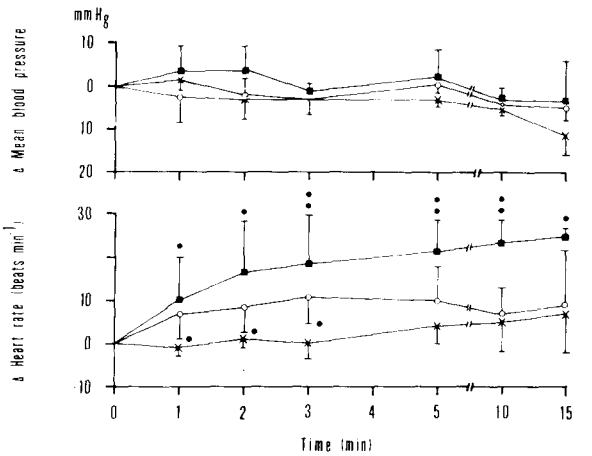

Fig. 2. Effect of substance P dissolved in ACSF (74.0 pmol ○——○, 222.9 pmol ■——■, ACSF ×——×) on mean arterial pressure and heart rate after local microinjection into the NTS (AP +0.46 ± 0.18, L 0.5, V 0.9) of urethane anesthetized rats. Mean ± S.D.; $n = 3$–5; •: $P < 0.05$; ••: $P < 0.01$.

Cardiovascular effects of capsaicin

A summary of the results obtained with microinjection of capsaicin (9.82 and 98.2 nmol) at zero level and (98.2 nmol) at the level of the obex is given in Fig. 3. The solvent of capsaicin in the volume of 0.2 μl did not evoke significant changes in blood pressure and heart rate after application at the same sites. Capsaicin administered at the level of the obex evoked hypotension (Fig. 3A) and bradycardia (Fig. 3C). The maximum effect occurred at about 0.5 min. The decrease in blood pressure and heart rate persisted for about 3 min. Local application of capsaicin in the dose of 9.82 nmol at zero level evoked hypotension persisting throughout the experiment (Fig. 3B). However, the hypotension evoked by the higher dose of capsaicin persisted only up to the fifth minute. The maximum fall in arterial pressure occurred

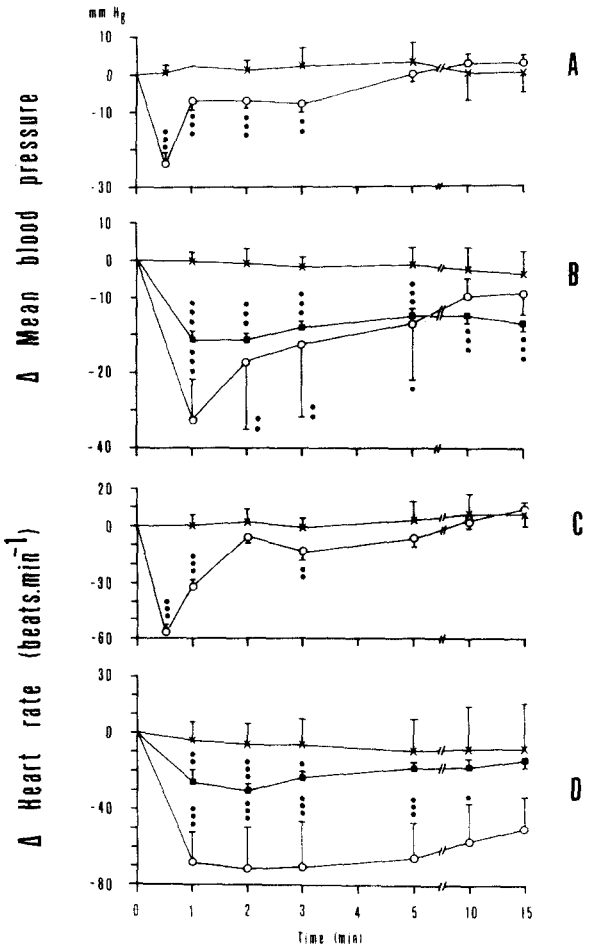

Fig. 3. Effect of capsaicin (9.82 nmol ■——■ and 98.2 nmol ○——○) and vehicle (×——×) on mean arterial pressure and heart rate after local administration into the NTS of urethane-anesthetized rats. Capsaicin and dissolvent were microinjected into the NTS at coordinates AP 0.0, L 0.5, V 0.9 (B,D) and AP +1.2, L 0.8, V 0.6 (A,C). Mean ± S.D.; $n = 5$–8; •: $P < 0.05$; ••: $P < 0.01$; •••: $P < 0.001$.

in the first minute (Fig. 3B). Bradycardia evoked by capsaicin (9.82 and 98.2 nmol) after its application at the zero level was dose-dependent (Fig. 3D). The maximum bradycardiac effect of capsaicin (98.2 nmol) was 78.3 ± 16.5 beats/min and it occurred at about 2 min. The hypotension and bradycardia after the higher dose of capsaicin at the level of the obex were of shorter duration and the maximum changes occurred earlier in comparison to changes evoked by capsaicin at zero level.

DISCUSSION

The microinjection of SP into 6 sites throughout the rostrocaudal distance of the NTS showed two sites as being most sensitive. The given localization of the hypotensive and bradycardiac actions of SP at the zero level (caudal tip of area postrema) and the level of the obex may have to do with (1) the distribution of terminals of baroreceptor fibers[7,24] located in the NTS close to the obex and in the commissural region, and (2) the presence of substance P in terminals of baro- and chemoreceptor afferents of the baroreflex arc in the NTS, as reported in recent studies[13,17].

Microinjection of SP into the caudal part of the NTS and 0.8 mm rostrally from the zero level, or into surrounding tissue had no effect on the cardiovascular system. Cardiovascular changes evoked by the administration of SP or capsaicin do not seem to be caused by mechanical damage of the tissue. Application of the solvent for capsaicin, of saline (except one site), or of ACSF, did not induce cardiovascular changes.

In order to rule out an action of microinjected SP primarily via interference with respiratory control, we performed a control experiment in urethanized, paralyzed rats maintained on a respirator. Administration of 74 pmol in the NTS at zero level (AP 0.0, L 0.5, V 0.9) and at the level of the obex (AP +1.2, L 0.8, V 0.6) caused a similar maximal decrease in mean arterial pressure and heart rate ($P < 0.01$) as shown for this dose of the peptide in Fig. 1, while saline had no significant effect at either site. The decreases for the two groups amounted 24.1 ± 12.0 and 23.0 ± 4.4 mm Hg, and 33.2 ± 17.6 and 30.0 ± 20.0 beats/min respectively (means ± S.D. of data of 5 rats in each group). Thus, SP injected in these two sites of the NTS appears to cause hypotension and bradycardia independent of respiratory control.

The role of SP in the regulation of the cardiovascular system at the level of the NTS has been studied previously[2,14,15,37], but the results of these studies are rather conflicting. One of the reasons for the controversial data may be the selection of different sites for application, as indicated by the present results. At the coordinate AP +0.42 ± 0.18, L 0.5, V 0.9 SP evoked only tachycardia. At other sites in the NTS, except at zero level and obex, it evoked changes in neither blood pressure nor in heart rate. Pronounced decreases in both parameters were recorded after microinjection of SP at the zero level and the level of the obex. Hypotension, bradycardia and decrease in

sympathetic nerve activity[15] were reported after application of SP into the NTS at the level of the obex of urethane anesthetized rats and cats. However, these changes were induced by doses which were approximately 60–200-fold higher and which were applied in a volume 12.5–37.5 times larger than used in our experiments.

Immunohistochemical studies[13,17] showed the presence of an SP-like immunoreactive material in baro- and chemoreceptor afferents entering the NTS in the IXth and Xth cranial nerves. Local application of SP into the NTS may evoke activation of the second order neurons of the baroreceptor reflex arc which are endowed with SP receptive sites[23,33]. Microinjection of SP in our experiments caused hypotension and bradycardia, or tachycardia without changes in blood pressure. Histological findings indicate a synaptic connection between SP-like immunoreactive nerve terminals with catecholaminergic and non-catecholaminergic neurons in the NTS[21,28,36]. The activation of different second-order neurons may explain the different effects of SP microinjected into the NTS at different coordinates.

Binding studies showed the existence of 3 classes[31] of tachykinin receptors in mammalian brain, i.e. SP, substance K (neurokinin A), and neuromedin K (neurokinin B) sub-types. Autoradiographic studies indicated that SP and substance K receptors are localized in the NTS in high concentrations[23,34]. However, the competition rank order for the tachykinins on the SP receptor is SP > physalaemin > substance K > eledoisin > kassinin > neuromedin K. In contrast to this rank order, SP has a much lower affinity for substance K receptors. SP administered into the NTS may possibly stimulate (dose-dependently) both these receptors, or only one. Up till now there is little information available about the functional role of these two tachykinins and their receptors in the NTS in connection with the regulation of the cardiovascular system. For further characterization of the role of tachykinins and their receptors in the NTS in connection with the regulation of the cardiovascular system, highly selective agonists and antagonists are needed.

A further key question concerns the activity of the endopeptidases which degrade SP via several routes[32]. It is known that the SP fragment SP_{1-7} has a potent antinociceptive activity[35] and also affects behaviour[10,18]. The degradation of [^{3}H]SP monitored in vitro in the guinea pig ileum and rat vas deferens[41] showed that after one minute more than 50% of the extracted tritium from both tissues was present in the form of metabolites. On the basis of these data we can expect that fragments of SP may also participate in changes of blood pressure and heart rate evoked by SP.

In the present study, an analog of SP, (D-Pro^2, D-Trp^{7,9})-SP, which is considered to act as an antagonist of SP, did not show any blocking effect in the dose used. These results are not in accordance with the antagonism found in peripheral systems[16,22] as well as in the locus ceruleus[9]. Engberg et al.[9] demonstrated that the above-mentioned analog of SP almost completely blocked the excitatory effect of SP on locus ceruleus neurons. They demonstrated that the SP-blocking action of the antagonist was short-lived. The effect of SP was blocked only by simultaneous application of the antagonist. In the light of these results, the 3-min interval between the application of the antagonist and of SP may have caused the ineffectiveness of the antagonist in the present experiments. However, the dose and the presence of different receptors in these structures may also play a role.

Capsaicin is an agent known to cause release of SP from primary afferent fibers[11]. Its microinjection into the NTS produced a cardiovascular effect comparable to that of SP, although the hypotension and bradycardia were slightly more pronounced and longer-lasting. Our observations may indicate that the hypotensive and bradycardiac effect of SP and capsaicin microinjected into the NTS may mimic the effect of SP released from primary afferents of the cranial nerves IX and X. However, further experiments with specific antagonists of SP are needed to confirm this assumption. A more detailed explanation of the capsaicin effect on the circulatory system after its local application into the NTS should also take into consideration the capacity of capsaicin to release 5-HT (ref. 4) and somatostatin[12] which are present in the NTS[25,29].

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