Substance P modulates nicotinic responses of intracardiac neurons to acetylcholine in the guinea pig

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Zhang, Lili, John D. Tompkins, John C. Hancock, and Donald B. Hoover. Substance P modulates nicotinic responses of intracardiac neurons to acetylcholine in the guinea pig. Am J Physiol Regulatory Integrative Comp Physiol 281: R1792–R1800, 2001.—Application of substance P (SP) to intracardiac neurons of the guinea pig causes slow depolarization and increases neuronal excitability. The present study was done to determine the influence of SP on fast excitatory responses of intracardiac neurons to ACh. Intracellular recording methods were used to measure responses of intracardiac neurons in whole mount preparations of atrial ganglionated nerve plexus from guinea pig hearts. Local pressure ejection of 100 μM SP (1 s) from a glass micropipette caused slow depolarization of all neurons (n = 38) and triggered action potential generation in 47% of the cells tested. Bath application of SP (0.5–100 μM) caused a dose-dependent depolarization of intracardiac neurons but rarely evoked action potentials, even at the highest concentration. However, such treatment with SP enhanced nicotinic responses evoked by local pressure ejections of ACh (10 mM, 10- to 100-ms duration) in 77% of intracardiac neurons studied (n = 52). A significant increase in amplitude of ACh-evoked fast depolarization occurred during treatment with 0.5 μM SP (13.0 ± 1.8 mV for control vs. 17.7 ± 1.9 mV with SP present, n = 7, P = 0.019). At higher concentrations of SP, enhancement of the response to ACh resulted mainly in action potential generation. However, responses to ACh were attenuated by SP in 15% of the intracardiac neurons studied. This attenuation occurred primarily during exposure to 10 and 100 μM SP and was manifest as a reduction in amplitude of nicotinic fast depolarization or inhibition of ACh-evoked action potentials. These findings support the conclusion that SP could function as a neuromodulator and neurotransmitter in intracardiac ganglia of the guinea pig.

Key Words: intracardiac ganglia; microelectrode recording; cholinergic neurons; sensory-motor nerves

THE TACHYKININ, SUBSTANCE P (SP), is present in a unique population of sensory-motor nerve fibers within the heart. These intracardiac nerve fibers arise from neurons located in the dorsal root ganglia and are activated during myocardial ischemia (6, 12, 32). The efferent actions of such sensory-motor neurons are mediated by tachykinins that are released from peripheral nerve processes (e.g., those in the heart), and afferent signals are transmitted by tachykinins that are released from central processes of the same neurons (6, 20, 24). SP-immunoreactive nerve fibers have a wide distribution within the heart and are particularly abundant in the intracardiac ganglia (33, 35). Many of the principal neurons of these ganglia are surrounded by varicose nerve fibers that are immunoreactive for SP (29, 33–35). There is also autoradiographic evidence that tachykinin receptors are localized to intracardiac ganglia of the guinea pig (11, 13) and dog (30). These anatomical findings support the concept that tachykinins could function as neurotransmitters at the intracardiac ganglia.

Administration of SP to isolated guinea pig hearts and anesthetized guinea pigs causes a prominent bradycardia that is blocked by atropine and potentiated by treatment with a cholinesterase inhibitor (10, 31). These observations demonstrate that the negative chronotropic action of SP is mediated by cholinergic neurons. Further evidence from isolated heart experiments suggests that cholinergic neurons may also be implicated in negative inotropic responses elicited by SP (4). Although these studies did not establish whether SP acted on cholinergic cell bodies located in the intracardiac ganglia or at their peripheral processes within the myocardium, autoradiographic experiments have detected specific binding of radiolabeled tachykinins to intracardiac ganglia and coronary arteries but not to atrial or ventricular myocardium (11, 13). Accordingly, SP appears to cause bradycardia by stimulating cholinergic cell bodies that are present within intracardiac ganglia.

Several investigators have used ganglionated nerve plexus preparations from guinea pig heart in electrophysiological experiments to evaluate the effects of SP on intracardiac neurons (8, 9, 17). These studies have established that SP has direct effects on such cells to cause slow membrane depolarization and increase neuronal excitability (8, 9, 17). In some cases, application of SP triggered bursts of action potentials (8, 12, 17). Slow depolarization similar to that produced by SP can be evoked by stimulation of nerve bundles in that...
preparation. These nerve-evoked responses were abolished by superfusion with Ca\(^{2+}\)-free buffer (8) and attenuated by treatment with a nonselective tachykinin receptor antagonist (17). Accordingly, it is likely that endogenous SP mediates at least a portion of the slow depolarization of intracardiac neurons evoked by nerve stimulation. Our recent study with isolated hearts provided further evidence that endogenous tachykinins can stimulate intracardiac cholinergic neurons (3). In these experiments, capsaicin was used to stimulate the release of tachykinins from sensory-motor nerve processes within the heart and a calcitonin gene-related peptide (CGRP) antagonist was used to block effects from CGRP that was coreleased with the tachykinins. A cholinesterase inhibitor was also present to amplify cholinergic responses. Under these conditions, capsaicin caused bradycardia that was attenuated by muscarinic receptor blockade or desensitization of tachykinin receptors. These findings implicate endogenous tachykinins and cholinergic neurons in the negative chronotropic response elicited by capsaicin.

The preceding evidence makes a strong case for a neurotransmitter role of SP within intracardiac ganglia, but the precise function of tachykinins at these sites remains unclear. One likely scenario that has been proposed is that SP and other tachykinins enhance the response of postganglionic parasympathetic neurons to vagal nerve stimulation (8, 12, 17). This speculation is supported by results from studies that evaluated the influence of SP on ganglionic transmission in sympathetic ganglia (15); however, the effect of SP on the response of guinea pig intracardiac neurons to ACh has not been reported. Accordingly, the principal aim of this investigation was to characterize interactions of SP and ACh in affecting neurons of the guinea pig intracardiac ganglia. As a byproduct of these studies, we also broadened our understanding of the separate effects of ACh and SP on intracardiac neurons.

**MATERIALS AND METHODS**

**Experimental preparation.** Male Hartley guinea pigs (250–350 g, n = 62) were anesthetized by intraperitoneal injection of 60 mg/kg pentobarbital sodium. The heart was rapidly excised and placed into a modified Krebs buffer containing 60 mg/kg pentobarbital sodium. The heart was rapidly excised and placed into a modified Krebs buffer (composition in mM: 121 NaCl, 25 NaHCO₃, 1.2 NaH₂PO₄, 5.9 KCl, 8 dextrose, 1.2 MgCl₂, 2.5 CaCl₂, equilibrated with 95% O₂–5% CO₂; pH 7.4–5–10°C). The atria were removed, pinned to the Sylgard floor of a custom recording chamber (20 ml) and continuously superfused (5 ml/min) with oxygenated buffer at 10°C. An Olympus SZ60 stereomicroscope and fiber optic illumination were used to isolate the atrial ganglionated plexus. Overlying atrial and vascular tissue were removed by dissection from the endocardial surface. The ganglionated plexus is embedded in a connective tissue membrane on the dorsal side of the heart (8, 14, 18, 27). After completion of the dissection, the preparation was allowed to equilibrate for 1 h while the temperature was gradually increased. Basic intracellular microelectrode recordings were made from intracardiac neurons with the temperature of the superfusate maintained at 34°C by a thermostatically controlled heater (Warner Instruments, Hamden, CT).

**Microelectrode recording.** Intracellular recordings from neurons were made using high-impedance (70–150 MΩ) borosilicate glass micropipettes filled with filtered 3 M KCl. Transmembrane potentials were measured in a current clamp configuration using an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA). After intracardiac neurons were impaled, they were allowed to stabilize for ~3 min before membrane properties were measured. Brief intracellular current pulses, triggered by a Grass S88 stimulator, were given through the recording electrode to characterize neurons. Intracellular current and potential signals were visualized using both analog and digital Tektronix oscilloscopes. Data were acquired and analyzed using a computer equipped with a Digidata 1200 analog-to-digital converter and the pCLAMP software suite (Axon Instruments).

**Drugs and drug administration.** ACh chloride and atropine sulfate were purchased from Sigma (St. Louis, MO) and SP from Phoenix Pharmaceuticals (Mountain View, CA). SP was dissolved in sterile saline, and aliquots of a 1 mM stock solution were stored at ~80°C. ACh and atropine were prepared fresh for each experiment. SP was administered by local pressure ejection from glass micropipettes (~5 μm tip bore) using a picospritzer (General Valve, Fairfield, NJ) or by bath application. Application of ACh was by local pressure ejection.

**Protocol for evaluating interactions between ACh and SP.** The design of these experiments was modeled after that used by Jiang and Dun (15) in their study of sympathetic neurons in the guinea pig inferior mesenteric ganglion. For these experiments, ACh (10 μM) was administered by local pressure ejection from glass micropipettes and SP was given by bath application. It is known that ACh can activate muscarinic receptors on intracardiac neurons to produce slow depolarization and/or slow hyperpolarization of the cell membrane (2). Such slow responses to ACh were prevented in this series of experiments by addition of 1 μM atropine to the superfusion buffer. Under this condition, ACh only evoked fast responses mediated by nicotinic receptors.

For each neuron, we first identified a duration of ACh pulse that produced a fast depolarization below the threshold for firing. Test pulses were separated by at least 25 s to avoid nicotinic receptor desensitization. After a suitable pulse duration (10 to 100 ms) was identified, ACh pulses of this length were applied repeatedly at 25- or 30-s intervals to evoke fast depolarizations of consistent magnitude. While such phasic applications of ACh were continued, the preparation was superfused for 2–6 min with buffer containing 0.5, 1, 10, or 100 μM SP. Applications of ACh continued during the washout period.

**Data analysis and statistics.** Only neurons with stable resting membrane potentials more negative than ~40 mV were included in the analysis. Whole cell input resistance was estimated from the slope of the line plotting membrane potential displacements from resting membrane potential against the amplitude of a series of hyperpolarizing pulses. Action potential duration was measured at two-thirds peak amplitude. Afterhyperpolarization duration was measured from the point at which the repolarizing potential crossed the level of the resting membrane potential to the point at which the potential had returned to one-half the peak amplitude of the afterhyperpolarization.

Data are expressed as mean ± SE (n = number of cells). Values for different groups were compared by using a paired or unpaired t-test (2 tailed) or by one-way ANOVA. Post hoc
comparisons after ANOVA were made using the Newman-Keuls procedure. \( P \) values < 0.05 were considered significant.

RESULTS

Passive and active membrane properties of intracardiac neurons. Basic membrane electrical properties and characteristics of action potentials generated by intracellular stimulation were determined for each recorded neuron. Table 1 summarizes these values for a representative population of cells that was subsequently used to determine responses to SP or ACh applied by pressure ejection. Neurons were further characterized by determining their response to a prolonged (1 s) intracellular depolarizing current pulse. Phasic neurons fired only one action potential during this pulse, whereas accommodating neurons fired multiple action potentials at a decreasing rate. Phasic neurons comprised 84% of the population in Table 1 and 75% of 175 intracardiac neurons evaluated in the entire study. Phasic and accommodating neurons did not differ in membrane properties, action potential characteristics, or afterhyperpolarization features (Table 1).

Responses of intracardiac neurons to pressure ejection of SP and ACh. Initial experiments were done to determine the responses evoked by local application of SP (100 \( \mu \)M) or ACh (1 mM) using a picospritzer. SP pulses of 1-s duration caused slow depolarization of all neurons evaluated (\( n = 38 \)) and triggered firing of action potentials in 47% of these cells (Fig. 1). Phasic and accommodating neurons did not differ regarding the maximum depolarization produced by SP (14 ± 1 mV for phasic cells, \( n = 31 \) ) vs. 12 ± 2 mV for accommodating, \( n = 7 ; P > 0.05 \) ), but SP evoked longer trains of action potentials in accommodating neurons (47 ± 10 action potentials per phasic cell, \( n = 14 \) vs. 116 ± 19 per accommodating cell, \( n = 4 ; P < 0.01 \) ). ACh pulses of the same duration as used with SP caused complex responses containing fast and slow components (\( n = 51 \) ). The slow component comprised a depolarizing response or a combination of depolarizing and afterhyperpolarizing changes in membrane potential. Only 35% of intracardiac neurons fired in response to 1-s pulses of ACh. The average number of action potentials (phasic + accommodating neurons) evoked by ACh was substantially less than the average for SP (8 ± 5 for ACh, \( n = 18 \) vs. 62 ± 11 for SP, \( n = 18 ; P < 0.0001 \) ). The influence of pulse duration on the response to pressure ejection of 1 mM ACh was subsequently evaluated in eight neurons (Fig. 2). Graded responses were evident in most of these experiments (Fig. 2), and 75% of these cells fired in response to two or more different pulse durations of ACh. Nevertheless, slow responses were evident with ACh pulses as short as 10 ms. Since slow depolarizing and afterhyperpolarizing responses to ACh are mediated by muscarinic receptors (2), atropine was used to block these effects in ensuing experiments.

Modulation of responses to ACh by bath application of SP. Repeated pressure ejection of ACh (10 mM, 10- to 100-ms pulse duration) at 25- or 30-s intervals caused fast depolarizations that were consistent in amplitude and subthreshold for generation of an action potential in most intracardiac neurons (Figs. 3 and 5). During such phasic challenges with ACh, SP was administered by bath application at one of four concentrations (0.5 \( \mu \)M, \( n = 18 \); 1 \( \mu \)M, \( n = 18 \); 10 \( \mu \)M, \( n = 11 \); and 100 \( \mu \)M, \( n = 5 \)). Bath application of SP caused a concentration-dependent slow depolarization of intracardiac neurons (\( F_{3,50} = 19.47, P < 0.0001 \) ). The per-

Table 1. Characterization of guinea pig intracardiac neurons

<table>
<thead>
<tr>
<th></th>
<th>Phasic (n = 102)</th>
<th>Accommodating (n = 19)</th>
</tr>
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<tbody>
<tr>
<td>Resting membrane potential, mV</td>
<td>−51.5 ± 0.5</td>
<td>−52.1 ± 1.1</td>
</tr>
<tr>
<td>Whole cell input resistance, MΩ</td>
<td>71.1 ± 3.8</td>
<td>68.4 ± 9.2</td>
</tr>
<tr>
<td>Action potential characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Threshold, mV</td>
<td>−37.6 ± 0.7</td>
<td>−37.7 ± 1.7</td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>67.6 ± 0.9</td>
<td>69.2 ± 2.4</td>
</tr>
<tr>
<td>Overshoot, mV</td>
<td>16.1 ± 0.9</td>
<td>17.1 ± 2.1</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>1.19 ± 0.03</td>
<td>1.15 ± 0.06</td>
</tr>
<tr>
<td>After hyperpolarization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>14.9 ± 0.3</td>
<td>15.5 ± 0.8</td>
</tr>
<tr>
<td>Duration, ms</td>
<td>110 ± 7</td>
<td>106 ± 11</td>
</tr>
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</table>

Values are means ± SE for each group. Neurons were classified as phasic or accommodating based on the spike pattern evoked by a 1-s depolarizing current pulse. No significant differences between groups were detected by unpaired \( t \)-tests.
The percentage of cells exhibiting slow depolarization in response to SP also increased with concentration of peptide (33%, 83%, 91%, and 100% at 0.5, 1, 10, and 100 μM SP, respectively). The largest depolarization occurred with 100 μM SP (10.9 ± 0.9 mV, n = 7). Bath application of SP rarely caused the generation of action potentials by itself as frequently occurred when 100 μM SP was given by local pressure ejection. However, bath application of SP did affect the response to ACh in 94% of the intracardiac neurons studied (n = 52). The remaining 6% exhibited no membrane response to SP (2 cells with 0.5 μM SP and 1 cell with 1 μM SP). The interaction between SP and ACh was manifest as augmentation of the response to ACh, attenuation of the response to ACh, or both of the later effects at different times during exposure to SP.

Potentiation of the response to ACh was most common (77% of neurons), and this effect occurred as either an increase in the amplitude of ACh-evoked fast depolarization (Fig. 3, A and B), conversion of the ACh-evoked response to action potential generation (Fig. 3, C and D), or a mixed response comprising larger ACh-evoked fast depolarization and at least one ACh-evoked action potential. Generation of at least one ACh-evoked action potential during exposure to SP was more common with 1 μM SP (72% of 18 cells evaluated) but occurred with a large percentage of...
intracardiac neurons at each concentration of the peptide (0.5 μM SP: 50% of 18 neurons; 10 μM SP: 55% of 11 neurons; 100 μM SP: 60% of 5 neurons). Within this population of neurons, multiple action potentials (usually 2–3) were often generated by single pulses of ACh (0.5 μM SP: 5 of 9 cells; 1 μM SP: 7 of 13 cells; 10 μM SP: 4 of 6 cells; 100 μM SP: 2 of 3 cells). Such responses usually occurred with at least two subsequent pulses of ACh during exposure to SP.

Amplification of ACh-evoked fast depolarization by SP was quantified by measuring the changes in membrane potential evoked by five pulses of ACh given before exposure to SP and changes evoked by pulses given during bath application of the peptide. A significant increase in amplitude of ACh-evoked slow depolarization occurred with seven neurons during treatment with 0.5 μM SP. For these cells, ACh depolarized the membrane by 13.0 ± 1.8 mV under control conditions and 17.7 ± 1.9 mV during exposure to SP (paired t-test, P = 0.019, n = 7).

For one intracardiac neuron with a very stable impalement, we also examined the effect of SP on responses to longer pressure ejections of ACh that evoked primarily a single action potential per pulse. During bath application of SP, this neuron fired multiple action potentials with each pulse of ACh (Fig. 4).

An inhibitory influence of SP on ACh-evoked fast depolarization was also encountered in a smaller population of cells (15% of 52 neurons studied). Attenuation of the response to ACh occurred in 4 of 11 neurons treated with 10 μM SP (and in 2 of 5 neurons treated with 100 μM SP) but rarely occurred in neurons exposed to 0.5 or 1 μM SP (1 of 18 neurons at each concentration). In one such instance, 0.5 μM SP reduced the magnitude of ACh-evoked fast depolarization without affecting the resting membrane potential (Fig. 5A). However, SP produced slow depolarization of the membrane in all other neurons that exhibited an inhibitory interaction between SP and ACh (Fig. 5B). A significant decrease in amplitude of ACh-evoked slow depolarization occurred with four neurons during treatment with 10 μM SP. For these cells, ACh depolarized the membrane by 16.4 ± 1.9 mV under control conditions and 11.5 ± 1.4 mV during exposure to SP (paired t-test, P = 0.012, n = 4). Finally, attenuation of ACh-evoked fast depolarization was occasionally observed in neurons following an initial interval of potentiation by SP. An example of this pattern of response is illustrated in Fig. 3D. The inhibitory action of SP in such cases occurred toward the end of superfusion with the peptide.

For one intracardiac neuron that exhibited an inhibitory interaction between SP and ACh, we were also able to evaluate the effect of SP on ACh-evoked firing. Bath application of 10 μM SP cause a slow depolarization of this cell and inhibited ACh-evoked firing for several minutes (Fig. 5C).

Effect of resting membrane potential on responses to ACh. Because SP caused a slow depolarization of the cell membrane, a series of control experiments were done to quantify the influence of membrane potential on the amplitude of ACh-evoked fast depolarization. Resting membrane potential was depolarized or hyperpolarized relative to the control value by injecting positive or negative current through the recording electrode. Current was adjusted to cause an absolute change in membrane potential that approximated the average depolarization produced by 1 μM SP. ACh was administered by local pressure injection every 30 s as in the preceding experiments. Figure 6 demonstrates that there is a significant correlation between the amplitude of ACh-evoked fast depolarization and the imposed change in resting membrane potential. Depolarization of the membrane attenuated the response to ACh, whereas hyperpolarization enhanced it.

DISCUSSION

The present study demonstrated that SP can potentiate excitatory responses to ACh in a majority of intracardiac neurons from the guinea pig. This potentiation occurred over a wide range of SP concentrations (0.5–100 μM) and was manifest as 1) an increase in the amplitude of ACh-evoked fast depolarization, 2) generation of one action potential or more by an ACh pulse that was subthreshold for firing in the absence of SP,
and 3) generation of multiple action potentials by an ACh pulse that evoked only a single action potential in the absence of SP. Augmentation of responses to ACh was not dependent on SP affecting the membrane potential but often occurred along with a slow depolarization. In a smaller number of intracardiac neurons, SP attenuated excitatory responses to ACh. This effect occurred predominantly with higher concentrations of SP (i.e., 10 and 100 μM) and was manifest as a reduction in the amplitude of ACh-evoked fast depolarization or an inhibition of ACh-evoked firing. Lastly, localized application of 100 μM SP caused many intracardiac neurons to fire trains of action potentials in the absence of ACh. These findings support the concept that SP may function as a neuromodulator and neurotransmitter within intracardiac ganglia of the guinea pig.

Previous investigators established that SP increases the excitability of guinea pig intracardiac neurons and triggers bursts of action potentials in some of these cells (8, 9, 17). Our findings expand on this work by establishing that SP can potentiate the response of intracardiac neurons to nicotinic stimulation by ACh. This potentiation showed a degree of concentration dependence in that 0.5 μM SP significantly increased the amplitude of ACh-evoked fast depolarization, whereas higher concentrations of SP often caused potentiation that was manifest primarily as generation of action potentials by ACh. We also quantified the percentage of intracardiac neurons that fired in response to pressure ejection of 100 μM SP and the number of action potentials evoked. Local application of SP caused significantly larger trains of action potentials in accommodating neurons than in phasic neurons. It is noteworthy that bath application of SP alone (0.5–100 μM) rarely evokes firing of intracardiac neurons while pressure ejection of 100 μM SP commonly evoked action potentials. Nevertheless, both methods of SP administration caused comparable levels of slow depolarization. Because the rate of SP delivery to the recorded neurons differs markedly between these methods, this factor could be important in determining the ability of SP to generate action potentials in intracardiac neurons.

**Fig. 5.** Inhibition of ACh-evoked responses by SP. For each panel (A-C), top traces show intracellular recordings from intracardiac neurons and bottom marks indicate times when ACh (10 mM) was given by local pressure ejection. The duration of ACh pulses was set to be subthreshold for action potential generation in A and B. ACh pulse duration was increased in C to generate action potentials in the absence of SP. The duration of ACh pulses was held constant within each experiment. Horizontal bars indicate intervals when SP was administered by bath application. A: bath application of SP did not affect membrane potential of this neuron but still reduced the amplitude of fast depolarization evoked by 20-ms ACh pulses. RMP = −50 mV. B: SP caused slow depolarization of this neuron and reduced the amplitude of fast depolarization evoked by 5-ms pulses of ACh given every 25 s. RMP = −49 mV. C: local application of ACh (28-ms pulse duration, 25-s interval) to this intracardiac neuron triggered firing that was inhibited by bath application of 10 μM SP but returned after SP was washed out of the system. SP also caused slow depolarization of this neuron. RMP = −56 mV.

**Fig. 6.** Effect of resting membrane potential on the amplitude of ACh-evoked fast depolarization. Graph shows that the amplitude of ACh-evoked fast depolarization changes in a linear fashion as resting membrane potential is altered. ACh pulses of constant duration were applied to intracardiac neurons every 30 s to evoke fast depolarizations of consistent magnitude. While such pulses were continued, the resting membrane potential was systematically altered by injection of positive or negative current through the recording electrode. Changes in the amplitude of ACh-evoked fast depolarization were calculated and expressed as a percentage of the average amplitude at the initial control membrane potential. Data points from 6 intracardiac neurons were evaluated by correlation analysis using Prism version 3.01 (GraphPad Software, San Diego, CA). The Pearson r = −0.86 for the pooled data (P < 0.0001).
SP is well known for its ability to stimulate various autonomic ganglia in the guinea pig and other species (1, 7, 15–17, 21, 23). The influence of SP on ganglionic function has been most thoroughly studied at the inferior mesenteric ganglia of guinea pigs (for reviews, see Refs. 24, 28). Application of SP to neurons within these ganglia caused slow depolarization and occasionally evoked bursts of action potentials. Importantly, SP potentiated the nicotinic depolarization evoked by nerve stimulation and by application of ACh (15). Also, there is evidence that endogenous SP has identical actions at these ganglia. Neuroanatomical work has established that mesenteric neurons are innervated by SP-containing collateral processes of sympathetic afferent neurons that project to the gut. Electrical stimulation of nerves that contain these afferent nerve fibers causes a slow excitatory postsynaptic potential (sEPSP) that is mediated in large part by SP. Stretch of the colon appears to be an important physiological stimulus that evokes SP-mediated sEPSPs in neurons of the inferior mesenteric ganglia (24, 28). Collectively, these observations support the conclusion that SP-containing afferent neurons mediate a short-loop reflex for control of intestinal activity (24, 28). Similar reflexes have been proposed for other tissues and may represent a common regulatory mechanism within the autonomic nervous system.

Other investigators have established that SP depolarizes guinea pig intracardiac neurons through tachykinin NK₃ receptor-mediated activation of a nonselective cation current (9). The same group recently demonstrated that phospholipase C (PLC) activity is required for activation of this current by SP (22). Accordingly, it is likely that SP causes concentration-dependent slow depolarization of intracardiac neurons by increasing PLC activity and the levels of downstream second messengers. It is not known if activation of PLC is also required for augmentation of fast excitory responses to ACh by SP. However, SP did enhance the response of a few intracardiac neurons to ACh without affecting the resting membrane potential. These observations suggest that membrane depolarization by SP is not required for it to enhance the neuronal response to ACh. On the contrary, responses to ACh were attenuated during the injection of depolarizing current into intracardiac neurons and augmented during the injection of hyperpolarizing current.

Although SP augmented the responsiveness of most intracardiac neurons to ACh, an inhibitory interaction was evident in a small percentage of ganglion cells. Such an inhibitory action might be attributed to depolarization of the cell membrane by SP and the resultant decrease in driving force for sodium ions to enter the neuron. Inhibition of ACh-evoked responses by SP has been reported to occur by this mechanism in a small number of myenteric neurons (15). However, we observed one clear instance in which SP markedly reduced the response of an intracardiac neuron to ACh without affecting the resting membrane potential. Accordingly, slow depolarization does not account for all cases of inhibitory interaction between SP and ACh.

There is strong biochemical and electrophysiological evidence that SP can act directly at nicotinic receptors to inhibit their function (1, 19, 25, 26). At frog sympathetic ganglia, SP produced slow depolarization that was mediated by tachykinin receptors and suppression of ACh-evoked responses by noncompetitive inhibition at the nicotinic receptor (1). More recent studies have evaluated the action of tachykinins at nicotinic receptors by using cells that express different combinations of receptor subunits (19, 26). This work has clearly demonstrated that SP acts as a noncompetitive inhibitor of nicotinic receptors. The apparent affinity of SP for the nicotinic receptors varied depending on their subunit composition and ranged from ~3 to 60 μM. In the present study, attenuation of ACh-evoked responses in guinea pig intracardiac neurons occurred most often during bath application of 10 or 100 μM SP. This finding is consistent with the conclusion that SP can inhibit ACh-evoked responses by binding to nicotinic receptors on guinea pig intracardiac neurons. Results from patch-clamp studies of rat intracardiac neurons provide convincing evidence that SP can attenuate nicotinic responses by this mechanism (5). Rat intracardiac neurons, in contrast to those of the guinea pig, are unaffected by application of SP alone and, therefore, appear to lack tachykinin receptors. However, local application of SP to these cells attenuated ACh-evoked whole cell currents in a concentration-dependent, voltage-independent, and reversible manner. Further studies of whole cell currents and outside-out membrane patches provided support for the conclusion that SP acted preferentially at nicotinic receptors comprising subunits that form large conductance channels (5). Significant attenuation of ACh-evoked responses was achieved with 3 μM SP in the latter experiments.

The use of 1-s pulses of ACh in our initial experiments was problematic, because it resulted in unusually long and complex responses that do not occur physiologically. The nature of these responses may be explained by the activation of both nicotinic and muscarinic receptors on the ganglion cells as well as possible activation of synaptic input to the recorded neuron through stimulation of prejunctional nicotinic receptors. Nevertheless, it is interesting that less than half of the intracardiac neurons fired an action potential in response to 1-s pulses of 1 mM ACh. Some ganglion cells likewise failed to fire when challenged with shorter or longer pulses of ACh, while others consistently fired action potentials when challenged with ACh over a range of pulse durations. This differential response pattern might be attributed to variations in the number of nicotinic receptors present on individual intracardiac neurons or variations in nicotinic receptor desensitization between ganglion cells. In this regard, it is noteworthy that most intracardiac neurons fired in response to shorter pulses of ACh during the second series of experiments when several...
pulse durations were tested to identify the pulse length required for evoking a subthreshold fast response.

Each neuron in the present study was classified as phasic or accommodating based on the response triggered by a 1-s depolarizing current pulse. With the use of this criterion, most intracardiac neurons were phasic cells. This observation is consistent with previous findings by other investigators (8). No clear-cut differences in responses to chemical stimuli were detected between phasic and accommodating neurons, with the exception that accommodating neurons fired longer trains of action potentials after pressure ejection of 100 μM SP. Whether accommodating neurons have a specialized function within the intrinsic cardiac nervous system is unknown.

Perspectives

It is well accepted that SP functions as an effenter neurotransmitter when released from sensory-motor nerve endings in the skin and those that innervate sympathetic ganglia within the gut. SP has been implicated as a mediator of axon reflexes at both of the latter sites. By analogy with these systems, SP may serve an identical function in the intrinsic cardiac nervous system where sensory-motor nerve fibers have a broad distribution within the heart. In this regard, it is possible that SP-containing sensory-motor neurons that innervate atrial and ventricular myocardium also have collateral processes that supply the intracardiac ganglia. If so, then short-loop reflexes (i.e., axon reflexes) might occur whereby stimulation ofafferent nerve fibers in the myocardium causes activation and release of SP from collateral projections to intracardiac neurons. Results from the present study suggest that endogenous SP, released within the intracardiac ganglia, could modulate the response of ganglion cells to vagal input and possibly trigger action potentials independent of ACh.

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REFERENCES


