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Substance P excites neurons in the gustatory zone of the rat nucleus tractus solitarius

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Whole-cell patch recordings of neurons in the rostral (gustatory) nucleus tractus solitarius (rNTS) were performed in a brain slice preparation from rat medulla. Neural responses to brief applications (10–45 s) of substance P (SP), via a constant superfusion apparatus, were recorded. SP transiently depolarized 80 of 117 (68%) rNTS neurons in a dose-dependent manner. Sub-micromolar concentrations of SP had potent excitatory effects, and the half maximal response occurred at 0.6 μM. The depolarizing effect of SP was accompanied by an increase in input resistance in 81% of the responsive neurons. The excitatory effects of SP persisted in low Ca^{2+} (0.2 mM) and high Mg^{2+} (12 mM) saline as well as in the presence of 2 μM TTX ($n = 5$ for each), suggesting direct postsynaptic action on the recorded neurons. SP also hyperpolarized 4 neurons (4%) and had no effect on 33 neurons (28%). Each of the 4 neurons which were hyperpolarized by SP showed a decrease in input resistance. A more detailed assessment of the types of neurons in the rNTS which respond to SP was also conducted. Neurons were separated into 4 electrophysiological groups on the basis of their repetitive firing pattern induced by a hyperpolarizing and depolarizing current injection paradigm. Neurons belonging to each of the 4 electrophysiological groups responded to SP. Eighteen neurons, which were filled with 1% biocytin during recording, were categorized as ovoid, multipolar or fusiform based on their morphological characteristics. SP excited all 3 morphological types of neurons in similar proportion. These results suggest that SP is an excitatory neurotransmitter in the rNTS. The effects of SP are not restricted to a particular neuron type defined either biophysically or morphologically. The implications of these results on the possible role of SP in processing gustatory and somatosensory information within the rNTS are discussed.

INTRODUCTION

Neurons in the rostral nucleus tractus solitarius (rNTS) receive gustatory and somatosensory afferent input from the chorda tympani, glossopharyngeal and superior laryngeal nerves[3,11,45,50]. The transmitters used by these nerves to relay gustatory information from taste buds to second order neurons in the rNTS are unknown. Substance P (SP)-immunoreactive fibers are associated with rat taste buds[9,27,29] and are found within the glossopharyngeal nerve[7,12] as well as within the rNTS[7,17], suggesting that SP may play a role in transmitting input from the tongue to the rNTS. Substance P (SP) receptors are located throughout the NTS[13,24], suggesting that SP may have physiological actions within this nucleus. To address the potential involvement of SP in the processing of gustatory information, we investigated the effects of SP on neurons in the rNTS using whole-cell recordings in a brain slice preparation of the rat medulla.

Previous whole-cell recordings have shown that neurons in the rNTS can be separated into 4 groups on the basis of their repetitive discharge pattern[5]. In *Group I* neurons, the repetitive discharge pattern initiated by membrane depolarization is changed to an irregular spike train by prior hyperpolarization. In *Group II* neurons, hyperpolarization either delays the occurrence of the first action potential or increases the length of the first interspike interval in the action potential train produced by membrane depolarization. In *Group III* neurons, hyperpolarization has little effect on the discharge pattern. In *Group IV* neurons,

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the discharge pattern consists of a short burst of action potentials that is often shortened by prior hyperpolarization.

Neurons in the rat rNTS have also been separated into morphological groups on the basis of somal shape, the number of primary dendrites and dendritic branching[20]. *Ovoid* cells have round to oval somas and two or more primary dendrites that are generally thin, and are sparsely and randomly branched. *Multipolar* cells have triangular or polygonal cell bodies with 3 to 5 primary dendrites. *Fusiform* cells have oval to elongate somas with two relatively thick primary dendrites that exit the cell body at opposite poles. A third smaller dendrite may also be present on fusiform cells. Multipolar and fusiform neurons have been reported to project to the parabrachial nucleus and caudal NTS[18,20,51], while ovoid cells contain gamma-aminobutyric acid (GABA) and are proposed to be interneurons[20]. In the current study, the effects of SP on these different electrophysiological and morphological groups of neurons in the rNTS have been investigated.

MATERIALS AND METHODS

Brain slice preparation

Brain slices containing the rostral 1.6 mm of the NTS were obtained from female Sprague–Dawley rats (Charles River) weighing 100–160 g, as previously described[4,5]. Briefly, rats anesthetized with sodium pentobarbital (50 mg/kg) were decapitated and the brainstem quickly removed, cooled and cut on a Vibratome into 400 μm thick coronal or horizontal slices. Before recording, the slices were incubated for at least 1 h in physiological saline at room temperature. The physiological saline contained (in mM): NaCl 124, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 26, KH_2PO_4 1.25, dextrose 25. The solution was gassed with 95% O_2 and 5% CO_2 to maintain the pH at 7.4. During recordings, slices were secured in a chamber with nylon mesh and continuously superfused with oxygenated saline flowing at 1.5–2 ml/min. By a system of valves, physiological saline containing SP (Sigma) at various concentrations ranging from 0.1 to 4 μM was superfused over the slice for 10–45 s. In some experiments, low Ca^{2+} (0.2 mM) and high Mg^{2+} (12 mM) saline or saline containing 2 μM tetrodotoxin (TTX; Sigma) was superfused over the slice for 4–7 min to block synaptic activity prior to SP application. Throughout the experiments the temperature was maintained between 31 and 33°C.

Electrophysiological techniques

Unpolished patch electrodes, pulled from 1.5 mm o.d. borosilicate filament glass (WPI, TW 150F-4) in two stages on a Narishige PP83 electrode puller, had tip resistances of 5–8 MΩ (bubble numbers 5.8–6.2)[26]. They were filled with a solution containing (in mM): potassium gluconate 130, HEPES 10, EGTA 10, $MgCl_2$ 1, $CaCl_2$ 1, ATP 2, and the pH was adjusted to 7.4 with KOH. The electrodes were positioned over the rNTS, which was easily identified when transluminated from below[4]. With the use of a hydraulic drive, the electrodes were advanced into the rNTS and whole-cell recordings were made using the technique of Blanton et al.[2], as previously described in detail[5]. Once a giga-ohm seal was formed and the patch ruptured, current stimulation protocols were performed and voltage data were acquired using the pCLAMP program (Axon Instruments) throughout the experiments. Current was injected into neurons using the bridge circuit of an Axoclamp 2A amplifier in current clamp mode. The indifferent electrode was a Ag-AgCl wire connected to the extracellular solution via an agar bridge. Neurons were separated into groups on the basis of their repetitive firing pattern induced by a 1200 ms, 100 pA depolarizing pulse, preceded by a −200 pA hyperpolarizing pulse of increasing duration (0, 50, 100 and 150 ms). Throughout the experiments membrane input resistance was measured using 100 ms, −100 pA hyperpolarizing current pulses at 0.15 Hz. These pulses caused a change in membrane potential along the linear phase of the current–voltage relationship and input resistance was measured at saturation. The average length of a recording period was 35 min.

Anatomical techniques

Since the boundaries of the rNTS were easily visible in the brain slice preparation, the position of the neurons was noted during recording. In coronal slices, the subdivision in which a neuron was located could be approximated at the time of recording. In horizontal slices, the medial–lateral position was clearly identified while recording but the dorsal–ventral position only could be approximated. The dorsal–ventral position was verified after cutting the horizontal slices into thinner sections (see below).

To determine the morphology of neurons which respond to SP, 18 neurons, all in horizontal slices, were filled with 1% biocytin[14] (Sigma), which was diluted in the pipette-filling solution and placed in the tip of the recording pipette. The neurons filled by diffusion and the biocytin did not affect the electrophysiological properties of the neurons. Following the experiments, the seal was broken by delivering several large hyperpolarizing pulses (−5 to −10 nA, 50 ms)[1], and the slices were removed from the recording chamber, placed on a piece of filter paper and fixed in 10% formalin for several days. Slices then were rinsed in 0.1 M phosphate buffer for 30 min, embedded in agar (4% in distilled H_2O) and cut into 100 μM thick sections on a Vibratome.

After rinsing the sections in phosphate buffer, they were incubated for 2 h in avidin-horseradish peroxidase (avidin-HRP; Vector Laboratories) at room temperature in the dark. The avidin-HRP was diluted 1:200 in phosphate-buffered saline containing 0.3% Triton-X (Sigma). The sections then were rinsed for 30 min and reacted with 0.025% diaminobenzidine and 0.01% H_2O_2 (Vector Laboratories) for 6 min. After rinsing, the sections were mounted on gelatin-coated slides, dried overnight and coverslipped using Krystalon mounting medium (Harleco Co.). The location of the neurons was verified and the neurons were assigned to morphological groups by visual inspection based on previously established criteria in the rNTS in rat[20] and hamster[8,50]. The neurons were reconstructed in 3 dimensions using the Eutectic Neuron Tracing System (Eutectic Electronics, Inc.).

Data analysis

A response to SP was defined as a noticeable change in membrane potential (>1 mV) concurrent with the application of SP, which usually returned toward baseline after wash out. The magnitude of responses was determined by comparing the membrane potential immediately prior to the application of SP to that during the peak of the response. The input resistance before and during application of SP was compared by analyzing the responses to a −100 pA, 100 ms hyperpolarizing current pulse applied at the appropriate times. Each concentration of SP could not be tested on every cell, therefore the effect of the highest concentration applied (usually at least 1 μM) was used to determine if the neuron was responsive. The effects of all applications of SP (in both responsive and unresponsive neurons) were combined in the dose–response curve (Fig. 3A) to avoid inflating the responses to the lower concentrations of SP. All data were analyzed and presented using the CLAMPFIT (Axon Instruments), AXOTAPE (Axon Instruments) and SYSTAT (SYSTAT, Inc.) programs. Responses of different neuron groups were compared by performing one-way analysis of variance with Tukey post-hoc tests. The effects of SP before and during application of low Ca^{2+} and high Mg^{2+} saline or TTX, and the changes in input resistance and latency caused by SP, were compared using a paired sample student T-test. The dose–response curve was fitted by nonlinear regression analysis using the function $\text{Response} = (C_1 \times \text{Concentration})/(C_2 + \text{Concentration})$, where C_1 is the maximum response to SP and C_2 is the concentration of SP

causing a half maximal response[43]. Data are presented as means ± S.E.M. and statistical significance is denoted as $P \leq 0.05$. A preliminary report of this study has appeared in abstract form[48].

RESULTS

Electrophysiological properties of rostral NTS neurons

The effects of SP were investigated on 117 neurons in the rNTS. The approximate location of 93 neurons recorded in coronal slices and 24 neurons recorded in horizontal slices is indicated in Fig. 1. Most cells (84%) recorded in coronal slices were located 1.6–2.0 mm rostral to obex. In horizontal slices, neurons were located throughout the rostral–caudal extent of the rNTS. The majority of the neurons (63%) were located within the rostral central and lateral subdivisions, regions which receive the central projections of the chorda tympani and glossopharyngeal nerves[11,50]. The remaining neurons were located within the medial (18%), ventral (12%) and dorsal (7%) subdivisions. Relatively few neurons (< 10%) were positioned within the extreme rostral portion of the NTS, an area which receives projections exclusively from the chorda tympani nerve[11,50]. The proportion of neurons receiving input from the chorda tympani and glossopharyngeal nerves could not be determined because most neurons (> 75%) were located within the region of overlap of their terminal fields[11,50]. The depth of the neurons within the slices ranged from 5 to 250 μm, with an average depth of 97 ± 6 μm. The orientation of the slices and the depth of the neurons within the slices did not effect the responses of the neurons to SP.

Neurons included in this study had a resting membrane potential more negative that −30 mV, an action potential overshoot greater than 10 mV and an input resistance greater than 150 MΩ. Resting membrane potentials ranged from −30 to −68 mV with a mean of −46 ± 1 mV. Action potential amplitude ranged from 45 to 105 mV (68 ± 1) with a mean duration measured at half amplitude of 1.8 ± 0.1 ms. Input resistance, determined from the response to a 100 ms, −100 pA hyperpolarizing current pulse, varied between 156 and 1020 MΩ and averaged 394 ± 14 MΩ. These values are similar to those obtained by us[5] and other investigators[25,40,42] using whole-cell recordings in brain slices. Most rNTS neurons (87%) were not spontaneously active. The passive membrane properties of the neurons did not effect their responsiveness to SP. For example, neurons with a resting membrane poten-

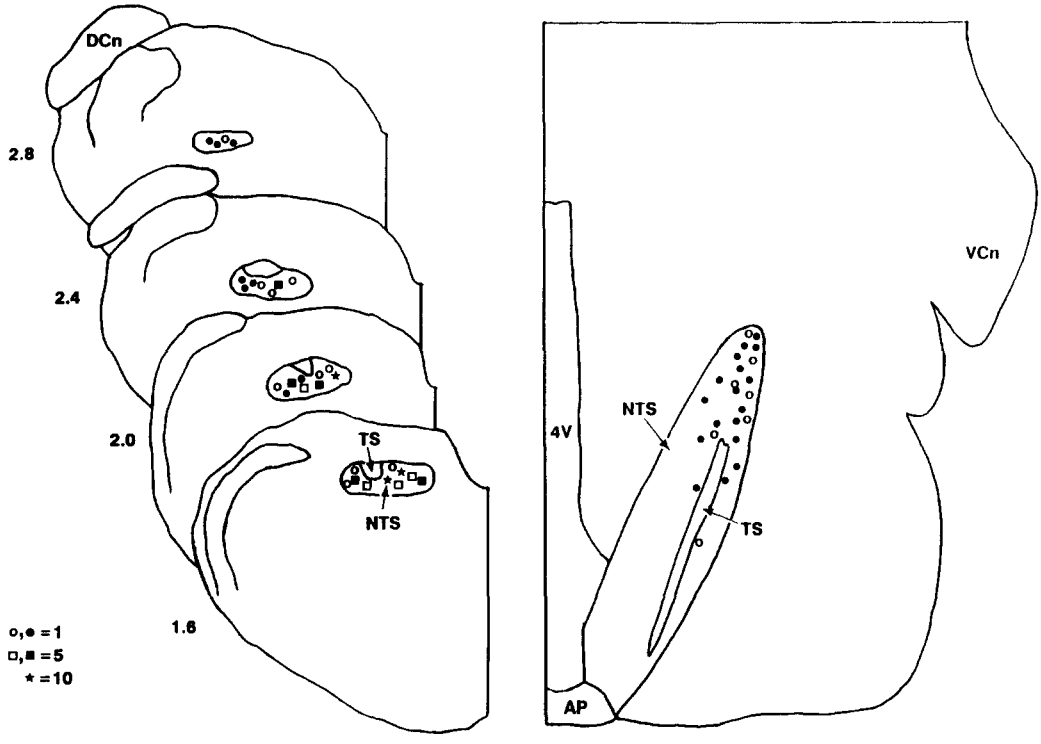

Fig. 1. Location of the 117 neurons recorded in the rNTS. 93 neurons are shown in diagrams of 4 coronal slices containing the rostral pole of the NTS (left) and 24 neurons are shown in a horizontal slice (right). For clarity, the location of the neurons is arbitrarily shown on the left in the coronal slices and on the right in the horizontal slice. Each symbol represents a different number of neurons as indicated on the lower left. Filled symbols indicate the position of neurons which responded to SP and open symbols indicate the position of unresponsive neurons. The numbers to the left of each coronal slice indicates the distance rostral to obex (in mm)[33]. AP, area postrema; DCn, dorsal cochlear nucleus; NTS, nucleus tractus solitarii; TS, tractus solitarius; VCn, ventral cochlear nucleus; 4V, 4th ventricle.

tial between −30 and −45 mV responded with similar magnitude and duration to SP than neurons with a resting membrane potential > −45 mV.

Substance P excites rostral NTS neurons

Of 117 neurons recorded in the rNTS, SP depolarized 80 (68%), hyperpolarized 4 (4%) and had no effect

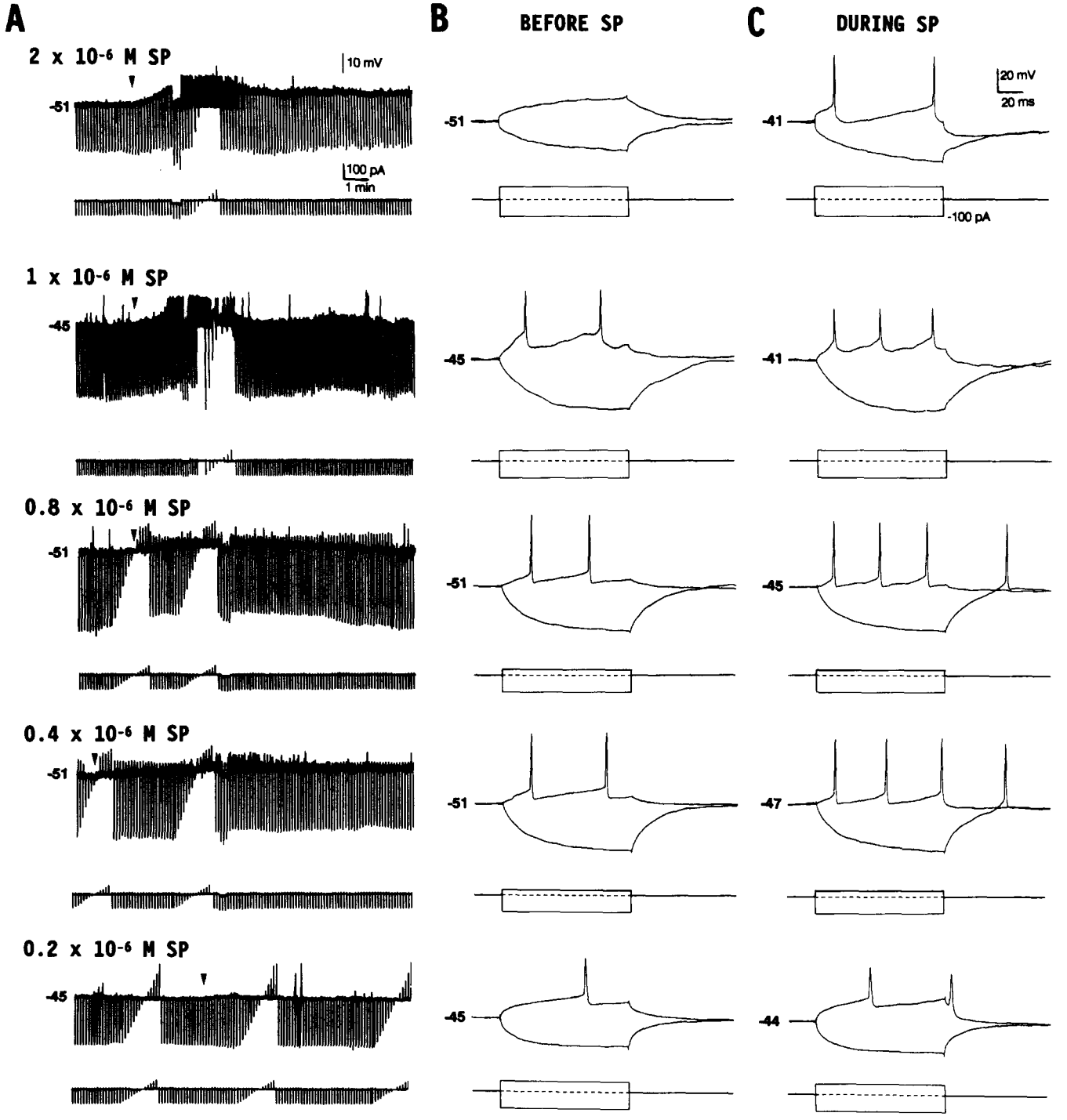

Fig. 2. A: effects of 5 concentrations of SP on the activity of 4 different rNTS neurons. The effects of 0.8 and 0.4 μM SP are in the same neuron. The upper trace of each pair shows the response to a 30 s application of the indicated concentration of SP. The beginning of the application of SP is indicated by the arrow head. In this trace, the action potentials are truncated (see B and C for full spikes). The resting membrane potential at the beginning of each trial is indicated to the left of each trace. The lower trace of each pair indicates the current injected. Constant current hyperpolarizing pulses (−100 pA, 100 ms duration, 5–6/s) were injected throughout the experiments to measure input resistance. The series of hyperpolarizing and depolarizing pulses were computer-generated for measurement of current-voltage relationships. During some responses, the neurons were hyperpolarized with direct current to the resting membrane potential to directly assess the change in input resistance. Notice the increase in input resistance induced by SP, especially apparent at 2 μM. B and C: responses of the same neurons to a hyperpolarizing (−100 pA) and a depolarizing (25, 50 or 75 pA) pulse (100 ms duration) before and during application of SP. Notice the greater number of spikes elicited by the depolarizing pulses and the reduced spike latency in the presence of SP.

on 33 (28%). The responsive and unresponsive neurons were not confined to one region of the rNTS (Fig. 1). Depolarizations were accompanied by an increase in input resistance in 81% of the responsive neurons. In neurons which responded to 4 μM SP, the input resistance was increased by 48 $\pm$ 14 MΩ ($P = 0.001$). In the remaining 19% of the neurons which were depolarized by SP, the input resistance did not change. Representative neuronal responses to different concentrations of SP are illustrated in Fig. 2. The duration of the responses always outlasted the duration of the application of SP. The response durations ranged from 1 to 28 min, and averaged 7 $\pm$ 1 min (all concentrations combined). Higher concentrations of SP usually caused neurons to become spontaneously active. In fact, 4 μM SP caused 62% of the neurons tested to fire action potentials. The depolarization caused by SP decreased the time to initiate the first action potential following a depolarizing pulse and increased the number of action potentials elicited during 100 ms depolarizing pulses (Fig. 2B and C). For example, the spike latency during a 150 pA depolarizing pulse was reduced by an average of 5.3 $\pm$ 1.2 ms by 2 μM SP ($P = 0.003$).

In 4 neurons, SP hyperpolarized the membrane potential by 9.2 $\pm$ 2.7% ($P < 0.05$; data not shown). Hyperpolarization was always accompanied by a decrease in input resistance, which averaged 95 $\pm$ 50 MΩ. The duration of the hyperpolarizing responses to 30 s applications of SP ranged from 2 to 8 min and averaged 4 $\pm$ 1 min.

The relationship between the percent change in membrane potential and the SP concentration is shown in Fig. 3A. Maximal responses (an 11% change in membrane potential) were elicited with 4 and 2 μM SP and half maximal responses occurred at a concentration of 0.6 μM. The duration of the responses also increased with increasing concentration of SP, ranging from 2.6 $\pm$ 0.5 min following application of 0.2 μM SP to 9.1 $\pm$ 1.7 min for 4 μM SP (Fig 3B).

The excitatory effects of SP were probably due to direct postsynaptic action on the recorded neurons, and not to indirect synaptic inputs, because the responses persisted in low Ca^{2+} and high Mg^{2+} saline and in the presence of TTX. The effects of low Ca^{2+} and high Mg^{2+} saline on the responses of rNTS neurons to SP are shown in Fig. 4. Although the action potentials elicited by SP were suppressed, the underlying depolarization was unchanged as compared to the responses in normal saline (Fig. 4A; $n = 5$; $P = 0.90$). In the low Ca^{2+} and high Mg^{2+} saline, SP continued to cause increases in input resistance as well as in the number of action potentials elicited by depolarization (Fig. 4B, C). The effects of TTX are shown in Fig. 5. Although the action potentials were completely blocked by TTX, the depolarizations produced by SP persisted and were similar to the responses in normal saline (Fig. 5A; $n = 5$; $P = 0.66$).

Neuronal types affected by substance P

A high percentage (62% to 82%) of neurons in all 4 electrophysiological groups were depolarized by SP (Fig. 6 and Table I). One neuron in each group was hyperpolarized by SP. The depolarizations were accompanied by an increase in input resistance and

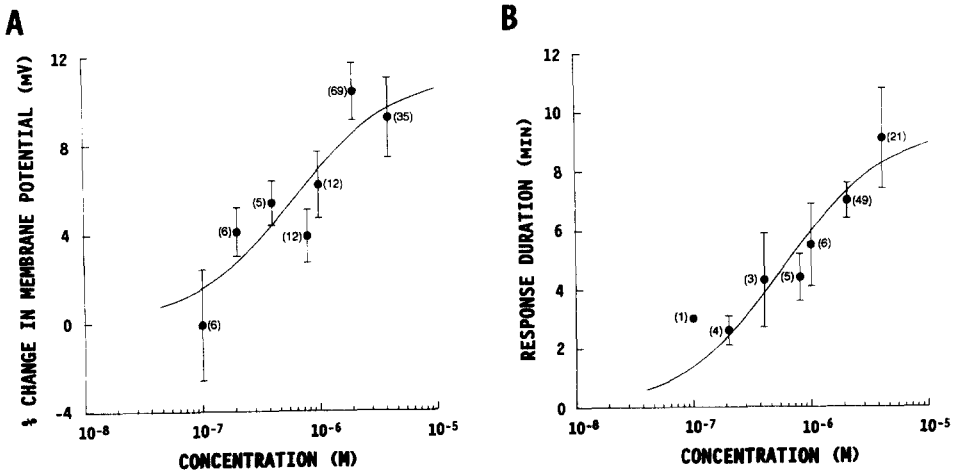

Fig. 3. A: dose–response curve showing the percent change in membrane potential as a function of the log of the concentration of SP. The number in parenthesis next to each point indicates the total number of applications of each concentration of SP. B: graph of the duration of the response as a function of the log of the concentration of SP. The number in parentheses next to each point indicates the number of neurons which responded to each concentration of SP. The curves were fitted by nonlinear regression analysis as explained in the Methods (in A, $r^2 = 0.74$; in B, $r^2 = 0.84$)

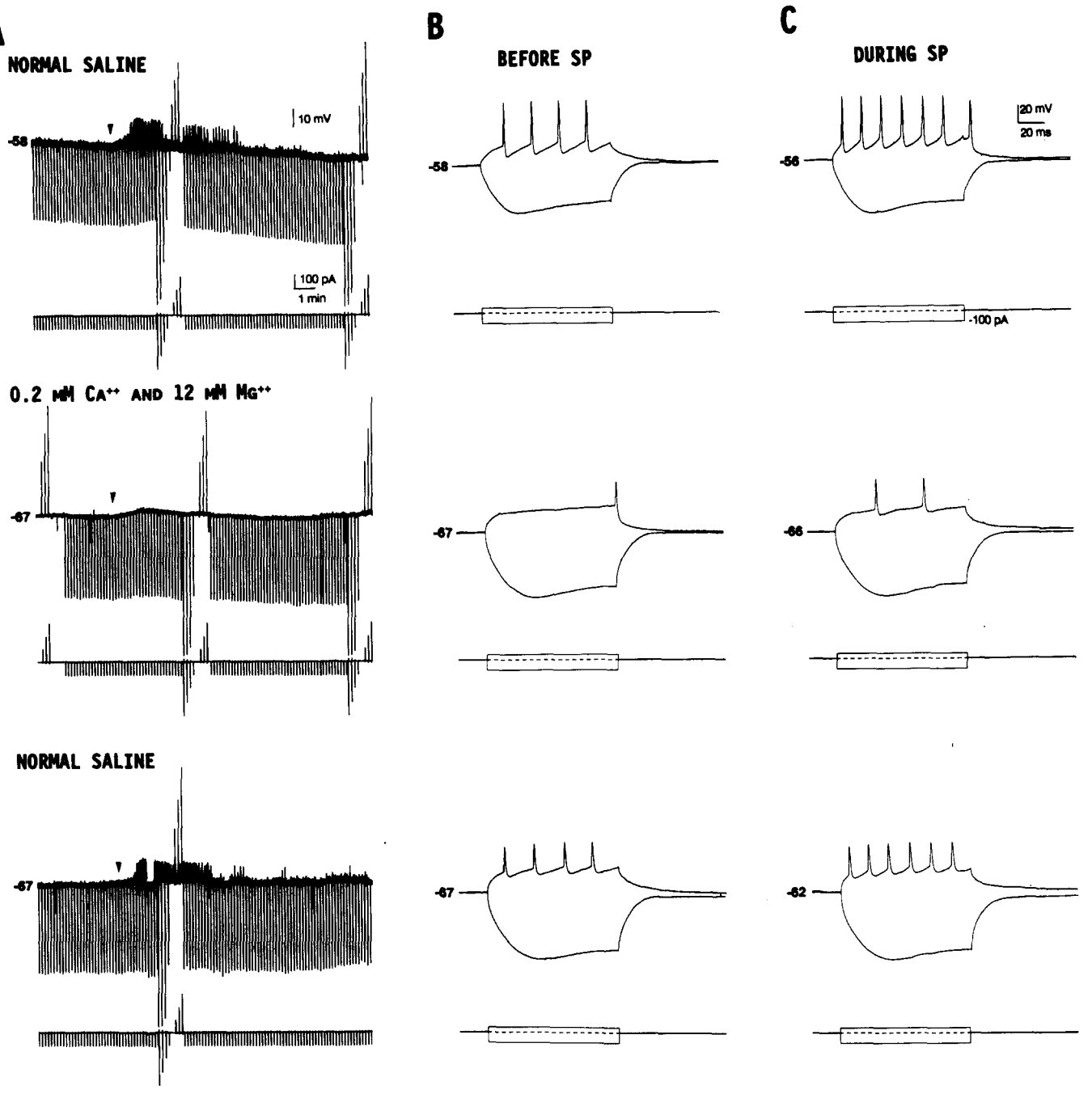

Fig. 4. A: responses of a single rNTS neuron to 10 s applications of 2 μM SP. The beginning of the SP application is indicated by the arrow head. The first and third applications were conducted in normal saline while the second was in low Ca^{2+} and high Mg^{2+} saline. The low Ca^{2+} and high Mg^{2+} saline was applied 5 min before and throughout the application of SP. Notice that while the action potentials were suppressed by the low Ca^{2+} and high Mg^{2+} saline, a similar underlying depolarization remained suggesting that SP acted directly on this neuron to elicit these responses. The membrane potential at the beginning of each trial is indicated to the left of each trace. B and C: responses of the same neuron to 100 ms pulses (−100 pA and 50 pA) before and during SP application in normal and low Ca^{2+} and high Mg^{2+} saline. SP increased the input resistance of this neuron during all 3 applications. Notice the wider and shorter action potentials in the low Ca^{2+} and high Mg^{2+} saline and the partial recovery after wash out.

caused an increase in the number of action potentials elicited by depolarizing current pulses (Fig. 6B). Responses of neurons in each group were similar except that Group II neurons responded with a slightly larger depolarization of longer duration than Group I neurons ($P < 0.05$; Table I).

Similar percentages of ovoid (73%; $n = 11$), multipolar (75%; $n = 4$) and fusiform (67%; $n = 3$) neurons were depolarized by SP (Fig. 7 and Table II). The characteristics of the responses of the different groups were similar except that the magnitude and duration of the depolarizations were slightly larger for the ovoid

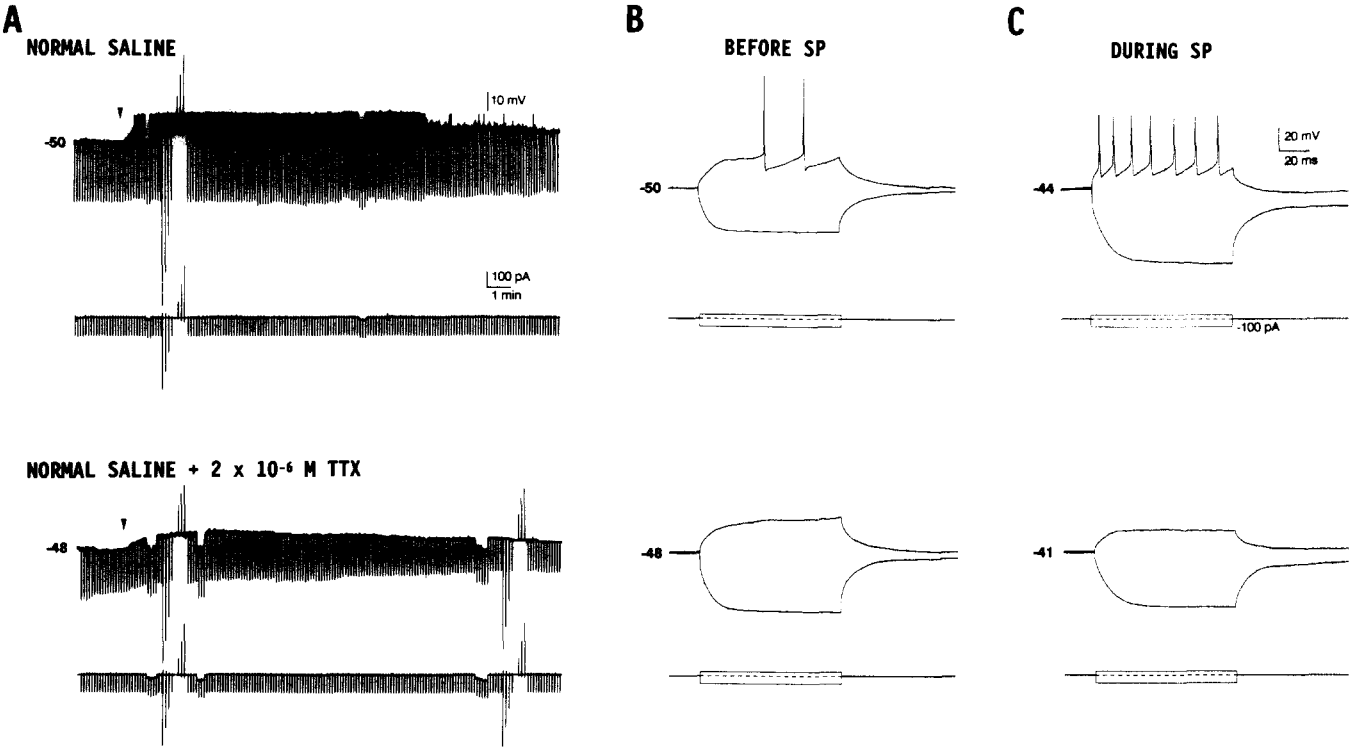

Fig. 5. A: responses of a single rNTS neuron to 15 s applications of 2 μM SP in normal saline and saline containing TTX. The TTX was present for 4 min prior to application of SP. The beginning of the SP application is indicated by the arrow head. This cell responded with very similar long-lasting depolarizations in both the normal and TTX-containing saline. This suggests that SP acted directly on this neuron. The membrane potential at the beginning of each trial is indicated to the left of each trace. B and C: responses of the same neuron to 100 ms pulses (-100 pA and 50 pA) before and during SP application in normal and TTX-containing saline. Notice the complete abolition of action potentials by TTX.

neurons than for the multipolar and fusiform neurons (not statistically significant, $P = 0.16$).

DISCUSSION

Substance P excites rostral NTS neurons

Substance P excited 68% of the neurons that we recorded from in the rNTS. The effects of SP were dose-dependent, with maximal responses occurring at concentrations of 2 and 4 μM and no responses at 0.1 μM SP. The robust effects of submicromolar concentrations of SP suggest that SP has potent physiological effects on rNTS neurons. The dose–response relationship is similar to that reported in the dorsal motor nucleus of the vagus (DMNX)[34], except that rNTS neurons may be more responsive to lower concentrations of SP. For example, while 0.4 μM SP elicited responses in 60% of the rNTS neurons tested, this same concentration was ineffective in the DMNX[34].

The effect of SP lasted an average of 7 ± 1 min and was usually accompanied by an increase in input resistance and a decrease in spike latency during stimulation. Excitatory effects of SP were probably due to direct postsynaptic action on the recorded neurons because the responses persisted in low Ca^{2+} and high Mg^{2+} saline and in the presence of TTX, procedures known to block synaptic transmission[35,37]. However, since these treatments were performed on only 10 neurons, it can not be concluded, with certainty, that SP had direct effects on all 80 of the responsive neurons.

A few neurons (4%) were hyperpolarized by SP. The inhibitory responses were accompanied by a reduction in input resistance and may have been due to direct action of SP on the recorded neurons, as previously reported in the DMNX[34]. This possibility was not investigated in the current experiments with low Ca^{2+} and high Mg^{2+} saline or TTX due to the infrequency of inhibitory responses to SP. It is also possible that another neurotransmitter, possibly gamma-aminobutyric acid (GABA), may have caused the inhibition. Therefore, the inhibitory effects of SP may have been indirect, resulting from activation of GABA-ergic interneurons[20]. Recently, GABA has been shown to have profound inhibitory effects using whole-cell recordings of neurons in slices of the rNTS[47].

The failure of 28% of the rNTS neurons to respond to SP could be due to several factors. First, neurons positioned deeper within the slices may not have been exposed to the expected concentrations of SP because of reduced access to the superfusion medium. This problem may have been caused by inadequate diffusion

TABLE I

Responsen of electrophysiological neuronal types

Values are means ± S.E.M. Data from all concentrations are combined. Only depolarizing responses are included, one neuron in each group was hyperpolarized by SP. % ΔRMP, percent change in resting membrane potential caused by SP.

Group	*n*	*% Responding*	*% ΔRMP (mV)*	*Duration (min)*
I	13	62	9.1 ± 2.5	3.5 ± 0.7
II	34	74	13.0 ± 1.6	9.2 ± 1.5 *
III	60	82	13.6 ± 1.3	6.2 ± 0.6
IV	10	70	12.3 ± 2.2	6.7 ± 1.9

* The responses of Group II neurons were significantly longer than the responses of Group I neurons ($P < 0.05$, ANOVA with Tukey post-hoc tests).

of SP within the slice or by binding, uptake or degradation of SP by neurons closer to the surface. However, this is unlikely because similar percentages of cells located at deep (100–250 μm) and superficial (5–100 μm) locations in the slices responded to SP. Second, neurons may have been unresponsive due to depletion of the G-protein-dependent second messenger system activated by SP[28]. This possibility is unlikely because all neurons were recorded under the same conditions, the composition of the pipette-filling solution was constant throughout the study and many cells still responded to SP after prolonged recording periods (> 1.5 h). Finally, the unresponsive neurons may not express SP receptors. Therefore, we conclude that although most rNTS neurons have SP receptors, not all do. This conclusion is supported by preliminary reports of recordings in slices of the hamster rNTS which demonstrated that 23% of rNTS neurons were unresponsive to SP[21,22].

Similar excitatory effects of SP have been reported in *in vitro* preparations of the caudal NTS[6,15], dorsal motor nucleus of the vagus[34], cholinergic cells of the

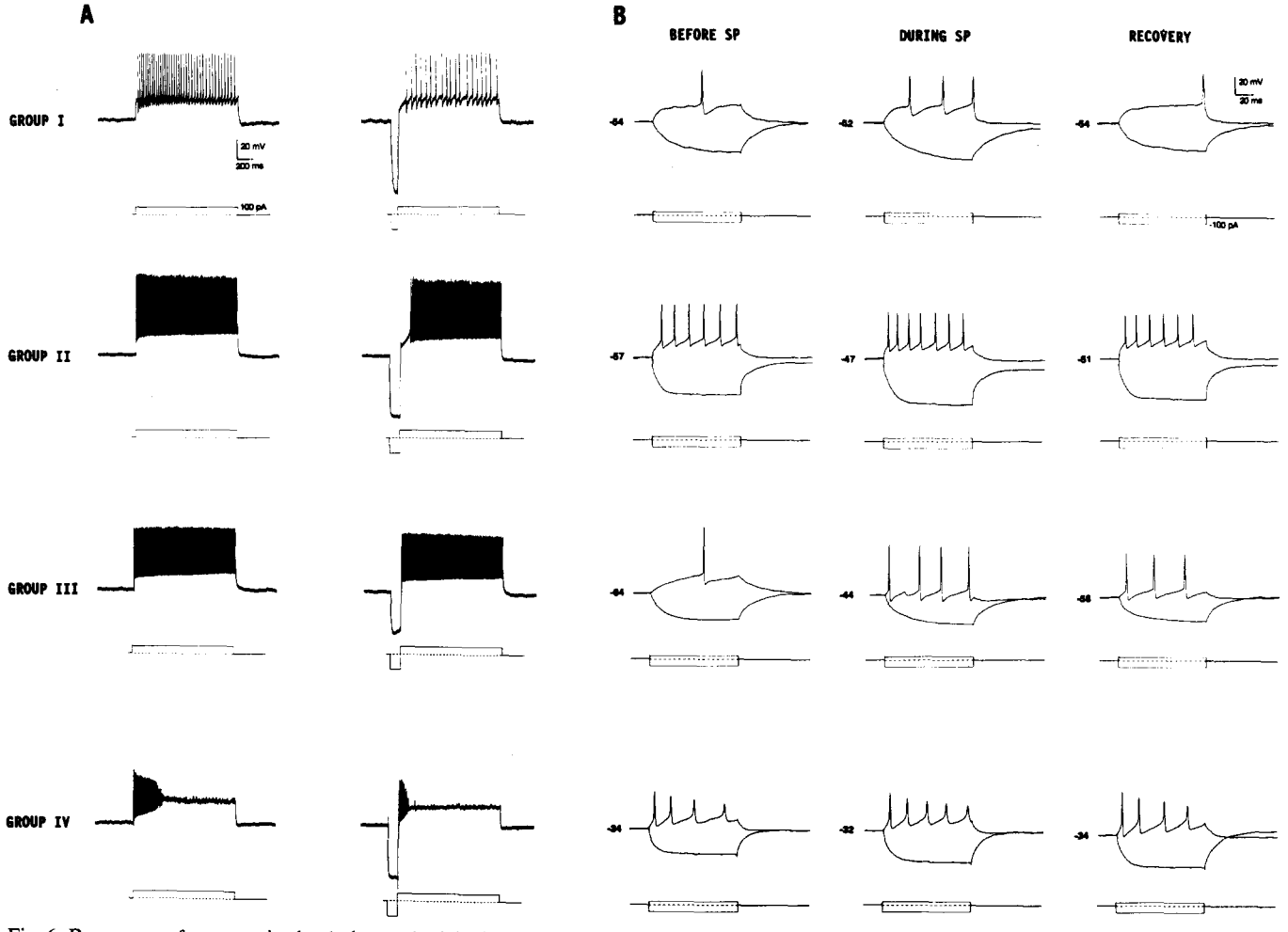

Fig. 6. Responses of neurons in the 4 electrophysiological groups to SP. A: repetitive firing discharge pattern of a neuron in each group elicited by a 1200 ms, 100 pA depolarizing pulse and by the same depolarizing pulse preceeded by a −200 pA hyperpolarizing pulse (100 ms duration for the group I neuron and 150 ms for the other neurons). B: responses of these neurons to a hyperpolarizing (−100 pA) and a depolarizing (50 pA) pulse before, during and after application of 2 μM SP. The membrane potential at the beginning of each trial is indicated to the left of each trace. Notice that SP depolarized all of these cells, increased the number of spikes induced by a depolarizing current pulse and increased the input resistance.

TABLE II

Responses of morphological neuronal types

Values are means ± S.E.M. All cells were tested with 2×10^{-6} M SP.

Group	*n*	*% Responding*	*% ΔRMP (mV)*	*Duration (min)*
Ovoid	11	73	17.1 ± 2.5	7.5 ± 1.6
Multipolar	4	75	12.8 ± 5.1	4.3 ± 0.9
Fusiform	3	67	11.5 ± 1.1	3.0 ± 0.0

basal forebrain[41,52] and trigeminal root ganglia[39]. In all but the latter experiments, the depolarization caused by SP was accompanied by an increase in input resistance, suggesting that the excitatory effects of SP resulted from the closing of ion channels. The effects of SP on neurons in the gustatory NTS are consistent with this mechanism.

Possible role of substance P in the rostral NTS

Substance P is prevalent in the peripheral nervous system and a role for SP in sensory systems, particularly nociception, has been proposed[16]. This proposal is based upon the presence of SP within unmyelinated and small myelinated primary afferent fibers, the depletion of SP from sensory fibers by capsaicin, the release of SP induced by noxious stimuli, and the excitatory effects of SP on nociceptive neurons in the dorsal horn of the spinal cord[16]. Since fibers immunoreactive for SP are associated with taste buds[9,27,29] and SP has been described within the glossopharyngeal[7] and possibly chorda tympani[29] nerves, the gustatory and pain systems may be similar in that SP may be released by the afferent fibers. The current studies support this hypothesis by demonstrating that neurons in regions of the rNTS which receive primary gustatory and somatosensory input[11,50,51], respond to SP.

Substance P input to the rNTS also may be derived from other brainstem areas. The caudal NTS contains many SP-immunoreactive cells[23] and some caudal NTS neurons may project rostrally within the nucleus[30]. Therefore, SP pathways may be part of an intranuclear network controlling visceral function. A portion of the SP input to the caudal NTS originates in trigeminal

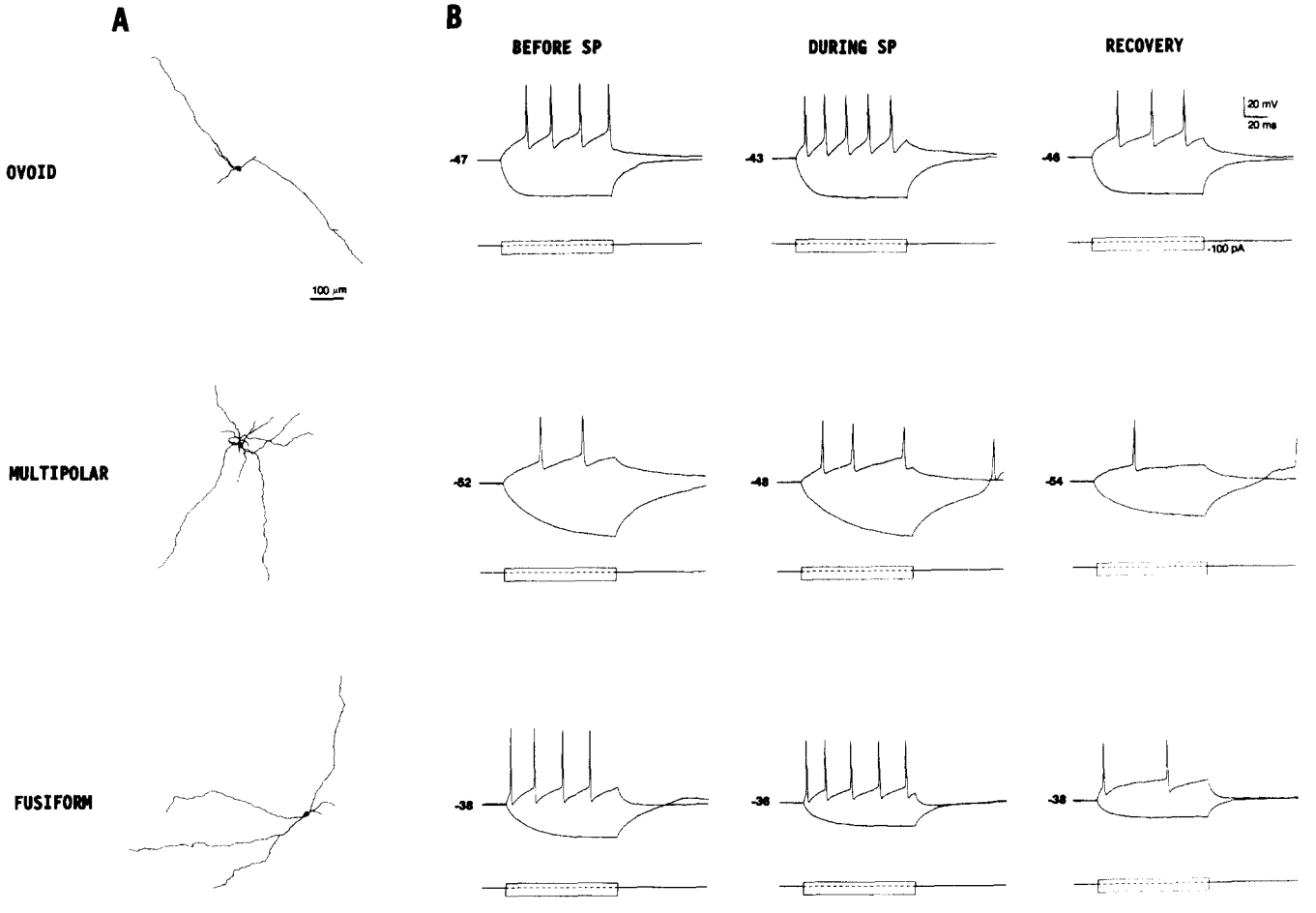

Fig. 7. Responses of neurons in the 3 morphological groups to SP. A: illustration of a neuron in each group. B: responses of the illustrated neurons to a hyperpolarizing (−100 pA) and a depolarizing (50 pA) pulse before, during and after application of 2 µM SP. The membrane potential at the beginning of each trial is indicated to the left of each trace.

sensory neurons of the spinal nucleus of the trigeminal nerve[38] and raphe nuclei[44], but comparable projections to the rNTS have not been described.

In addition, SP-immunoreactive neurons are located throughout the central nervous system[10,23,49], including several regions known to project to the rNTS. Combined anterograde and retrograde tracing studies have demonstrated that cells in the medial and lateral prefrontal cortex, bed nucleus of the stria terminalis, central nucleus of the amygdala, hypothalamic paraventricular nucleus, perifornical nucleus, arcuate nucleus and posterolateral areas of the hypothalamus project to the rNTS[46,54]. Neurons containing SP and mRNA encoding SP are located in all these areas except the hypothalamic paraventricular nucleus[10,23,49], suggesting that SP input to the rNTS may arise from these descending pathways. Since the critical double-labeling studies have not been conducted, it is not known whether the SP-immunoreactive cells in these areas project to the rNTS. Nevertheless, it can be speculated that the SP pathways may be part of a network which regulates visceral function by influencing NTS neurons. Each of the regions which project to the rNTS potentially receive indirect input from the rNTS through the parabrachial nucleus[19,30,31,36] and gustatory thalamus[32]. For example, the central amygdala[31] and gustatory neocortex[19] receive input from the parabrachial nucleus and project back to the rNTS. The forebrain structures and their projections may be important in taste discrimination and taste-guided behaviors[53]. In addition, the descending connections of these areas may regulate homeostatic functions of the NTS influencing visceral and consummatory function during stress or other emotional states.

In our whole-cell patch recordings, the effects of SP are not restricted to any of the electrophysiological[5] or morphological[8,20,50] groups of neurons previously identified in the rNTS. Therefore, a role for SP cannot be defined solely on the basis of the neuronal types within the rNTS. However, there are slight differences in the characteristics of the responses of neurons in the various electrophysiological and morphological groups. For example, Group II neurons responded to SP with larger depolarizations of longer duration than Group I neurons. Since the function of the electrophysiological groups has not been determined, implications for the role of SP remain unknown. It is hypothesized that Group III neurons may primarily be projection neurons, summing synaptic inputs and reliably transmitting this input to other CNS areas[5]. If this hypothesis is valid, then SP may have profound effects on the output of the rNTS. This proposal is substantiated by the fact that 75% of the multipolar neurons and 67% of the fusiform cells, the neuron types which project to the parabrachial nucleus and caudal NTS[18,20,51], respond to SP. Our morphological analysis also revealed that 73% of the ovoid cells, which are presumed GABA-ergic interneurons[20], respond to SP. Therefore, in addition to influencing potential projection neurons, SP effects interneurons, and thereby may also alter the activity of local neuronal circuits within the rNTS.

Conclusion

The current study indicates that SP has potent excitatory influences on neurons in the rostral (gustatory) NTS. Therefore, SP may be important for processing gustatory and somatosensory information within this nucleus. Taste buds and nerves relaying input from the oral cavity contain SP, suggesting that SP may play a role in the transmission of sensory information to the rNTS. In addition, SP fibers in the rNTS may originate in several forebrain structures, and therefore, SP may mediate hypothalamic or limbic influences on the function of the rNTS. Because all 4 electrophysiological and 3 morphological types of rNTS neurons respond to SP, it is proposed that SP influences both projection neurons and local circuit neurons within the rNTS; and therefore SP may have profound effects on information processing within this nucleus as well as on transmission of this information to other brain areas.

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