Wang, Xueyong and Donald Robertson. Substance P-induced inward current in identified auditory efferent neurons in rat brain stem slices. J. Neurophysiol. 80: 218–229, 1998. The effects of substance P (SP) on whole cell currents were studied in neurons of the medial olivocochlear efferent system (MOCS) in the ventral nucleus of the trapezoid body (VNTB) of brain stem slices from neonatal rats. Each neuron was identified by retrograde labeling with Fast Blue injected into the cochlea. Bath application of SP (0.1–10 µM) reversibly induced an apparent inward current in 49 of 63 labeled neurons when voltage clamped at near resting voltages. This apparent inward current was consistent with the SP-induced membrane depolarization observed in current-clamp mode. The SP-induced change in current was dose dependent with a half-maximal response dose of 200 nM. It was mimicked by [Cys³,⁶, Tyr⁴, Pro⁹]-SP, a neurokinin (NK1) receptor selective agonist, whereas [Succinyl-Asp⁶, MePhe⁸]-SP 6–11 (Senkide), a NK3 receptor agonist, had no detectable effect. The SP effect was not blocked by 10⁻⁶ M tetrodotoxin (TTX) and persisted when the perfusate contained 30 mM tetraethylammonium (TEA) or 100 µM Cd²⁺ or was in a 0-Ca solution. In a TTX-containing solution, SP caused a voltage-dependent decrease of membrane conductance, and the SP-evoked current reversed at a potential at around −105 mV. The predicted K⁺ equilibrium potential was −93.8 mV under the experimental conditions. The SP-induced inward current was attenuated by 66% when the perfusate contained 3 mM Cs⁺. We conclude that the apparent inward current is partly caused by SP decreasing an outward current normally maintained by the inward rectifier K⁺ channels in these cells. In the presence of Cs⁺ solution in the recording pipette and with a perfusate containing 3 mM Cs⁺, 0.1 mM Cd²⁺ and 10⁻⁶ M TTX, a residual SP-induced inward current was observed at test voltages ranging from −120 to 40 mV. This subcomponent reversed its polarity at −20 mV. This inward current was reduced substantially (but not abolished) when all NaCl in the external solution was replaced by TEA-Cl. The results indicate that SP also opens an unknown cation channel, which the available data suggests may be relatively nonspecific. The results suggest that MOCS neurons are subject to modulation by SP, which depolarizes the cell membrane by decreasing the activity of inward rectifier K⁺ channels as well as concurrently activating a separate cation conductance. It also was found that in MOCS neurons responsive to both SP and norpinephrine, the norpinephrine effect was abolished by TTX, suggesting that an interneuronal population excited by norpinephrine converges selectively onto SP-sensitive MOCS neurons in the VNTB.

**METHODS**

**Slice preparation**

Wistar rats of 5–10 days postnatal age were anesthetized with ether and 0.1–0.5 µl of a 1% aqueous suspension of Fast Blue was injected into the cochlea (Wang and Robertson 1997b). Rats were allowed to recover from the operation and were kept with their mothers for 1–5 days. Brain stem slices were prepared using a variation of a method described previously (Robertson 1996). Briefly, the animals were decapitated, and the brain stem with attached cerebellum was removed quickly and placed in ice cold artificial cerebrospinal fluid (ACSF). A block of tissue was cut into 300- to 350-µm sections with a Vibratome (series 1000). Slices (2–3) containing the VNTB were incubated in ACSF for ≥1.5 h before recording was commenced. The composition of
ACSF was (in mM) 130 NaCl, 3 KCl, 1.2 KH₂PO₄, 20 NaHCO₃, 2.4 CaCl₂, 1.3 MgCl₂, and 10 d-glucose, and pH was 7.4 after equilibration with 95% O₂-5% CO₂.

**Electrical recordings**

Whole cell patch-clamp recordings were made from Fast Blue-labeled neurons within the VNTB using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA). Output data were low-pass filtered at 5 kHz and collected through a DigiData 1200 interface using pClamp software 6.0 (Axon Instruments). Patch pipettes were pulled from borosilicate glass (Clark Electromedical Instruments, Reading, UK, 1.5 mm OD, 0.86 mm ID) with a Brown-Flaming P87 puller. Electrodes had tip resistances ranging from 2 to 4 MΩ when filled with a routinely used solution, referred as Glu-K solution, which contained (in mM) 115 K-gluconate, 35 KCl, 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, 2 MgSO₄, 10 ethylene glycol-bis(β-aminoethy) ether)-N,N,N’,N’- tetraacetic acid (EGTA), 2 Na-ATP, and 0.3 Na-GTP, pH 7.3. In a few experiments, variations of this solution were used including a Glu-Cs solution in which K-gluconate and KCl were replaced by 150 mM Cs-gluconate and a KCl-150 solution in which K-gluconate and KCl were replaced by 150 mM KCl. Before the experiment, 0.5–1 mg/ml Lucifer yellow (potassium salt, Sigma Chemical, St. Louis, MO) was added to the pipette solution. Seal formation between the tip of the electrode and the membrane of Fast Blue labeled neurons was directly visualized under a fluorescence microscope (×400; Leitz, Wetzlar, Germany). On establishing whole cell configuration, series resistance was compensated maximally with amplifier settings, and stable whole cell currents could be recorded for periods ranging from 15 min up to 2 h. A successful recording was confirmed and validated if Lucifer yellow filled the cell after the membrane patch was ruptured and if the membrane potential was more negative than −55 mV. All experiments were conducted at 25°C, which was maintained by pre-warming the perfusates. During the experiment, the slice was perfused constantly on both sides at a rate of 3–6 ml/min with a standard external solution, which was ACSF with the addition of tetrodotoxin (TTX; 10⁻⁶ M). All drugs were added to the contents of separate reservoirs at the final concentrations and applied to the slices by switching reservoirs. The time required for the new solution to travel from its reservoir to the chamber was 15 s to 1 min. When tetraethylammonium (TEA) and CsCl were used, the equivalent amount of NaCl was reduced to maintain ionic strength and osmolarity.

Clamped membrane voltages have been corrected off-line for junction potential, which was measured according to Neher (1992). For pipettes filled with the Glu-K solution, the junction potential correction was 7 mV in both ACSF and 30 mM TEA external solution. For pipettes filled with the Glu-Cs solution, junction potential corrections were 2 and 5 mV in ACSF and 130 mM TEA external solution, respectively. In the case of pipettes filled with 150 mM KCl solution, a correction of 4 mV was applied in ACSF.

**Chemicals and statistics**

SP and NE, were from Sigma Chemical. [Cys⁶⁶Tyr⁹Pro⁹]-SP and [Succinyl-Asp⁶, MePhe⁸]-substance P (Senktide) were from Auspep, (Parkville, Australia). The above drugs were dissolved in distilled water at concentrations of 10⁻⁴ M, stored in aliquots at less than −30°C, and diluted in ACSF immediately before use. Lucifer yellow, TEA, EGTA, ATP, GTP, TTX, CsCl, CaCl₂, and BaCl₂ were from Sigma Chemical. Fast Blue was from Dr. G. Illing, GMBH (Gross-Umstadt, Germany).

Unless otherwise stated, results are expressed as means ± SE (n), where n refers to number of neurons. Data measurements and analysis were performed with Clampfit (Axon Instruments). All statistics and curve fitting were done by using Sigmaplot (Version 3.0). All comparisons between means were performed using Student’s t-tests.

**RESULTS**

**SP-induced inward current**

Whole cell currents were examined from 63 Fast Blue-labeled neurons located in the VNTB. When the slice was perfused with the standard external solution, bath application of SP (0.1–5 μM) induced a slow inward current at a holding potential of either −55 or −75 mV in 45 of 63 cells tested. Typically, the inward current reached its peak in ~1 min and was followed by a slow recovery to the control level within ~3 min (e.g., Fig. 1A). The response was dose dependent. By measuring the peak amplitude of SP-induced inward currents at different doses applied while the cell was held at −55 mV, a half-maximal response dose of 0.2 μM was estimated (Fig. 1B).

Under the same condition, applying (Cys³⁶Tyr⁹Pro⁹)-SP, a neurokinin (NK1 type) receptor agonist (0.1–1 μM) also induced a similar response (n = 5), whereas Senktide, a neurokinin (NK3 type) receptor agonist, had no detectable effect on whole cell currents of five cells tested (Fig. 1A). These results were consistent with our previous report using intracellular microelectrode recording techniques (Wang and Robertson 1997a, 1998) and suggested that the inward current was mediated via neurokinin receptors of the NK1 type.

During prolonged application (>2 min) of SP at 0.5 and 1 μM, a gradual decay of the inward current after reaching its peak was observed, indicating a slow desensitization of the response. An example is shown in Fig. 1C. Empirically, we found that the best reproducibility was achieved when SP was applied for <1 min with ≥5 min washout in between two successive applications. As a precaution, all repetitive responses presented in this paper were obtained after at least a 10-min washout.

**Evidence that SP decreased the inward rectifier**

To investigate the ionic nature of the inward current induced by SP, we applied SP while recording the whole cell currents with a voltage protocol that allowed both the steady-state currents at the holding potential and inward and outward currents induced by a voltage step to be monitored. This protocol is illustrated in Fig. 2A. Voltage-dependent Na⁺ channels were blocked with 10⁻⁶ M TTX, shown in Fig. 2A by the blocking of transient inward currents evoked by depolarizing pulses. The existence of these rapid, transient inward currents before TTX application may indicate action potentials that escape the voltage clamp. Such escaped spikes did not occur in all cases, but their occurrence does indicate that the control of membrane voltage may not be good in all cases [probably as a result of the large soma size and extensive dendritic tree of MOCS neurons in slices (cf. Wang and Robertson 1998)] and quantitative measurements need to be treated with caution in these cells. Nonetheless, the results clearly show that the application of TTX, while blocking the transient inward currents evoked by depo-
larizing pulses, had no detectable effect on the slow inward current induced by 0.1 μM SP in the same cells (Fig. 2A).

Similar measurements of membrane currents were made when the perfusate contained 30 mM TEA as well as TTX. We found that there was no significant reduction of the SP-induced inward current under these circumstances (Fig. 2B) even though TEA did appear to cause some reduction in the voltage-dependent K+ currents evoked by depolarization. The absence of an effect of TEA on the SP effect is in marked contrast to the action of this K+ channel blocker on the excitatory action of NE in these neurons, which we have previously reported to be the result of a decreased K+ conductance. In the case of NE, the effect is blocked partially by 30 mM TEA (Wang and Robertson 1997a,b).

In a small number of cells, the voltage protocol used in Fig. 2 also revealed an additional action of SP that comprised a small reduction in the outward current evoked by depolarizing pulses (e.g., Fig. 8). We did not study this infrequent and inconsistent phenomenon further in the present series of experiments.

The I-V relationship of SP responses was investigated in detail using either successive voltage steps (Fig. 3, A and B) or voltage ramp (Fig. 3C). Both techniques revealed similar results and showed that SP induced an inward current accompanied by a reduction in membrane conductance. The SP-induced inward current reversed at around $-105 \pm 4$ mV ($n = 5$), which is reasonably close to the theoretical K+ equilibrium potential ($-93.8$ mV) in our system. There seemed to be little or no involvement of a change in anion conductance in the response to SP because the equilibrium potential for Cl- was $-33$ mV in our experimental configuration. Furthermore, in two cases when the SP responses were recorded with pipettes that contained 150 mM KCl-solution instead of the usual 35 mM, the reversal potential of the SP-induced current was virtually unchanged ($-108$ and $-98$ mV in each case; Fig. 3D).

Similar SP responses to those described above were obtained in three cases when the slice was bathed in a solution containing 0.1 mM Cd2+ to block voltage-dependent Ca channels or 4 mM Mg2+ with Ca2+ omitted. In the example shown in Fig. 4, the same cell showed similar responses to SP in the standard external solution, in the presence of 0.1 Cd2+, and in a 0 Ca2+ solution. Thus calcium influx does not

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**Fig. 1.** A: whole cell currents recorded from 1 neuron, at a holding potential ($V_h$) of $-55$ mV. Bath application of 0.5 μM substance P (SP; top) and NK1 agonist (bottom) induced an inward current, whereas [Succi-nyl-Asp6, MePhe8]-SP6–11 (Senktide; middle) had no effect. Resting membrane potential of the cell ($V_m$) was $-59$ mV. B: dose-response curve for SP-induced inward currents. Peak inward currents were measured at a holding potential of $V_h = -59$ mV after bath application of SP for 30 s. fit to the rectangular hyperbolic function $I_{SP} = C_{SP} \times I_{max}(K_d + C_{SP})$, where $I_{SP}$ is obtained by subtraction of the peak inward current from the baseline current, $C_{SP}$ is the concentration applied, $K_d$ is the dissociation constant (half-maximal response dose), and $I_{max}$ is the predicted maximal inward current induced by SP. Number of experiments at each concentration indicated in parentheses. Estimated $K_d$ and $I_{max}$ were $0.20 \pm 0.05$ μM and $-64.74 \pm 4.0$ pA, respectively. C: desensitization of SP responses. Whole cell current responses to bath application of SP 0.5 μM for 40 s (top) and for 3 min (bottom) at $-55$ mV. A decrease in amplitude of SP-induced inward current occurred during prolonged application. All of these experiments were carried out with the slice perfused in a standard external solution and the recording pipette filled with a K-Glu solution.
appear to contribute significantly to the SP-induced inward current in MOCS neurons.

To further explore the mechanism(s) by which SP exerts its excitatory effect, we carried out experiments in which the bathing solution contained 3 mM CsCl which blocks the inward rectifier in these cells (ref. Wang and Robertson 1997a,b). In the example shown in Fig. 5A, it is clear that 3 mM Cs causes a substantial reduction in the SP-induced inward current. When measured from a holding potential of −55 mV, the SP-induced inward current was attenuated on average by 65.9 ± 10.7% (n = 5). Similar reductions of the SP-induced responses by 3 mM Cs also were recorded with Cs-solution filled pipettes (n = 4) as summarized in Fig. 7.

Measurements of I-V characteristics in the presence of 3 mM Cs showed that, under these conditions, SP appeared
FIG. 3. A: whole cell currents elicited by successive voltage steps recorded before (a) and after (b) bath application of 0.2 μM SP. SP-induced current (c) was obtained by subtracting b from a. · · ··, 0 current level. Slice was perfused in a standard external solution containing TTX, and the pipette was filled with K-Glu solution. Recording sampling interval was 0.75 ms. Voltage steps used shown in (d). B: data from a–c plotted as I–V relationships. Current measurements were taken at the end of each trace (means of ~40-ms segments). ○, before SP; ●, after SP; —, subtraction showing SP-induced current. Reversal of SP-induced inward current occurs at about ~100 mV. C: whole cell currents evoked by voltage ramp plotted against voltage. Pipette was filled with Glu-K solution. Slice was perfused in a standard external solution. Inset: subtracted current with more sensitive vertical scale, indicating reversal potential of ~99 mV (—, linear regression fit to the data around the reversal potential). Note that reversal potential of SP-induced inward current obtained by this method is similar to that in B, obtained using voltage steps. D: ramp voltage-evoked currents from another cell recorded before and after application of 0.2 μM SP with a pipette filled with KCl solution. Slice was perfused in a standard external solution. Subtracted current record was noisy but linear regression suggests a reversal potential of ~108 mV. Subtracted currents in C and D were subjected to smoothing using 3 points adjacent average method.

to increase the membrane conductance, and there was an accompanying positive shift of the reversal potential of the SP-induced current to ~0 mV (Fig. 5, B and C). Thus the residual SP-induced inward current observed in the presence of Cs appears to be produced by a different mechanism from the major component. The latter can probably be attributed to the reduction by SP of a Cs-sensitive, TEA-insensitive, inward-rectifying K+ conductance that is active around resting values of membrane potential in MOCS neurons.

Evidence that SP activates a cation conductance

The residual response to SP seen in MOCS neurons after blockage of the inward rectifier was further investigated with the recording pipette filled with Cs-gluconate solution (with Cs+ replacing K+) and the slice perfused with a modified standard solution containing 3 mM Cs+ and with 10^{-6} M TTX and 0.1 mM Cd^{2+} to block voltage-gated Na+ and Ca^{2+} currents. Under these conditions, SP induced a small inward current in four of five cells tested. The average peak inward current was ~16.2 ± 3.7 pA at ~55 mV (n = 4) (Fig. 7). Under the same experimental conditions, the I–V relationships showed that the remaining inward current activated by SP became larger at more negative potentials, reversed at a potential around +20 ± 4.4 mV (n = 7), and also was accompanied by an increase in membrane conductance (Fig. 6, A and B).

The above result indicates that, in addition to a reduction
FIG. 4. Lack of Ca dependency of SP-induced response. Effect of SP (0.5 μM) on whole cell currents was tested while the cell was perfused with the standard external solution containing 2.4 mM Ca²⁺ or 2.4 mM Ca²⁺ and 0.1 mM Cd²⁺ or 4 mM Mg²⁺ with Ca²⁺ omitted. Recording pipette was filled with a K-Glu solution. Representative segments of current traces are shown top, and their corresponding locations are indicated by the letters (see Fig. 2 legend for details of experimental protocol).

Relationship to action of NE

In a previous voltage-clamp study, we have shown that NE induces an inward current in a majority of MOC neurons by decreasing an outward K⁺ conductance (Wang and Robertson, 1997a,b). We also have shown, using intracellular microelectrode recordings from the VNTB without prelabeling techniques to positively identify cells by their connectivity, that some neurons have their excitability altered by more than one neurotransmitter, in some cases both NE and SP (Wang and Robertson 1997c). We decided to revisit this issue in the present voltage-clamp study in which we could record selectively from MOCs neurons with the prelabeling technique. We were interested to investigate the similarities and differences between the excitatory actions of NE and SP.

In slices perfused in a standard external solution without TTX, three of a total of seven cells tested showed inward current responses to both drugs. However, when we recorded from cells in slices perfused with a solution containing TTX (10⁻⁶ M), we could not find any cells in a total of 16 that showed dual responsiveness. In the case of just one cell, we managed to record the response to both NE and SP before and after addition of TTX to the perfusion solution. The result of this experiment is shown in Fig. 8. Like a number of others, this cell also showed transient inward currents induced by depolarization that were blocked by TTX. Unlike most other cells, in this example, SP also produced a significant drop in the depolarization-induced outward current. This cell responded to both NE and SP with a large inward current in the absence of TTX. However, when TTX was added (10⁻⁶ M), the response to SP remained unaltered and the response to NE was abolished. Taken together, the results suggest that MOCs neurons are responsive to both NE and SP only under conditions where action potentials are present within the slice circuitry. Also observed in the one example where complete data were obtained (Fig. 8) was the appearance of spontaneous excitatory postsynaptic currents (EPSCs) in the presence of norepinephrine that were abolished by TTX. This apparent association between fast EPSCs and a slow excitatory current in the presence of norepinephrine is difficult to explain. The small sample size, and the fact that bursts of spontaneous EPSCs were occasionally seen under normal conditions, does not allow us to say whether the result was merely a coincidence in this particular case.

DISCUSSION

The present study demonstrates that SP induces an inward current in MOCs neurons in the rat brain stem. This inward current, which our evidence suggests is mediated via neurokinin NK1 receptors, results in an excitatory depolarizing response, which previous work using microelectrode implantations has shown to result in an increase in the effective-
ness of a depolarizing stimulus (Wang and Robertson 1992). SP has been reported to exert an excitatory effect in neurons in a number of regions of the CNS, and the ionic mechanisms involved have been found to vary in different neuronal preparations (see Otsuka and Yoshioka 1993 for review). Our data reveal at least two separate ionic mechanisms underlying the SP-induced inward current in MOCS neurons. A given MOCS neuron, if it is sensitive to SP, seems to possess both components to the evoked inward current. The decrease in membrane conductance, the reversal potential of the inward current, the lack of effect on this of changes in Cl⁻ gradient, and the effect of external Cs⁺, all indicate that the predominant mechanism is a decrease of an inward rectifying K⁺ current that is active around normal values of membrane potential. A similar mechanism has been described in cultured basal forebrain cholinergic neurons (Stanfield et al. 1985; Yamaguchi et al. 1990), in locus coeruleus neurons (Koyano et al. 1993; Shen and North 1992), and in dorsal vagal neurons (Martini-Luccarini et al. 1996). In a previous study, we showed that many MOCS neurons have their excitability increased by NE, also through the inactivation of a K⁺ channel (Wang and Robertson 1997a–c). However, in the case of NE, the effect can be blocked by TEA, and the K⁺ channel involved is evidently distinct from that which is regulated by SP.

In a small number of neurons (e.g., Fig. 8), we saw an additional effect of SP (decrease in outward current evoked by depolarization) that we did not investigate further. It seems to us that a decrease of a leak K current might be involved in such cases as has been reported in cultured celiac ganglia neurons (Vanner et al. 1993), but without further experiments, we do not feel justified in describing this rarely observed phenomenon further.

The evidence for a K⁺ involvement rests largely on the reversal potential for the SP effect being close to the calculated equilibrium potential for K⁺ but there are a number
FIG. 6. A: whole cell currents elicited by successive voltage steps recorded before (a) and after (b) bath application of 0.2 μM SP. Slice was perfused with a standard external solution that also contained 3 mM CsCl and 0.1 mM CdCl₂. Pipette was filled with a Cs-Glu solution. I-V curve (c) was constructed by plotting the currents measured at the end of each traces against voltage. d was obtained by subtracting currents after SP from control currents. Estimate of reversal potential (19.4 mV) was obtained by linear regression. Dotted line in a and b indicates 0 current level. B: whole cell currents evoked by voltage ramp before and after application of 0.5 μM SP under the same experimental condition as in A. SP-induced current reversed at ~20 mV. C: voltage ramp-evoked currents from another cell recorded under the same condition as in A except that all external NaCl (130 mM) was replaced by TEA-Cl. SP (0.5 μM) still induced a sizable inward current. SP-induced current reversed at around ~5 mV.

of difficulties in making such a quantitative comparison. The equilibrium potential for K⁺ was calculated from the ionic concentrations in the pipette and external solutions, but for strict accuracy, ionic activities should be used. In the case of the reversal potential itself, the magnitude of the SP-induced currents was small, introducing uncertainties in the measurement. In addition, the measured voltages had to be corrected using estimates of junction potentials. Even allowing for these uncertainties, however, the estimated reversal potentials did seem to be consistently more negative.
than the Nernst $K^+$ potential by 5–15 mV. One possible explanation may be that the receptors for SP are located on distal dendrites and the clamp voltage at these distant sites may not be well controlled by current injection in the cell soma. On balance, given all these factors, it would be remarkable if the reversal potential for the SP effect were exactly equal to the Nernst $K^+$ value. We feel that the large negative value obtained, the effect of Cs and the fact that changes in the Cl$^-$ gradient had little effect mean that the case for $K^+$ being involved is very strong.

After blocking the inward rectifying $K^+$ current, the inward current evoked by SP was not fully suppressed in MOCS neurons. The small residual inward current had a reversal potential shifted to more positive values (~0 mV) and was accompanied by an increase in membrane conductance. The behavior of the reversal potential in the presence of various concentrations of Na$^+$, TEA, and Cs$^+$ in either the bathing medium or the pipette suggests that the underlying mechanism of the residual SP-induced current is the activation of a relatively nonselective cation channel(s) with different permeabilities to Na$^+$, K$^+$, Cs$^+$ and possibly also to TEA. Such nonselective SP-activated conductance has been demonstrated in neurons of the locus coeruleus (Koyano et al. 1993; Shen and North 1992), spinal dorsal horn (Murase et al. 1989), dorsal root ganglia (Inoue et al. 1995), and neostriatum (Aosaki and Kawaguchi 1996).

The intracellular signaling mechanisms whereby SP binding to NK1 receptors leads to the inactivation of one channel type and the opening of another, both of which result in increased cell excitability, still have to be investigated in these MOCS neurons. SP has been reported to cause a rise in intracellular Ca$^{2+}$ in some tissues. Our results show that if Ca$^{2+}$ is involved in the SP effects in MOCS neurons, influx of extracellular Ca$^{2+}$ through the cell membrane is not required. In locus coeruleus neurons, evidence has been presented that the action of SP on the inward rectifier is mediated by a G protein, whereas the increased cation conductance appears to use a different, unknown mechanism (Koyano et al. 1993).

**Relation to NE responsiveness**

Our results also reveal interesting relationships between the responses of MOCS neurons to SP and NE that permit

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**FIG. 7.** Summary of SP-induced inward current in different ionic environments. Average peak amplitudes of inward currents induced by 0.5 μM SP at a holding potential of −55 mV were compared under experimental conditions indicated. Number of experiments in each group indicated in parentheses. With K-Glu solution in recording pipette, the SP-induced currents were −51.0 ± 6.9 pA in the standard external solution (Std), −47.4 ± 11.7 pA in presence of 30 mM TEA, and −17.6 ± 6.1 pA when 3 mM Cs$^+$ was present (the difference between the Std and TEA and Cs groups yielded $P > 0.05$, and $P < 0.01$, respectively, when compared with Student’s $t$-test). With Cs-Glu solution in recording pipette, the SP-induced currents were −46.2 ± 6.5 pA in the standard external solution, −16.2 ± 3.7 pA in presence of 3 mM Cs$^+$, and −8.5 pA in presence of 3 mM Cs$^+$ and 130 mM TEA (replaced NaCl; both the latter groups, when compared with the Std group yielded a $P < 0.01$ on a Student’s $t$-test). There was no significant difference between the amplitude of the SP responses recorded in the 3 mM Cs$^+$ 3 groups using either K-Glu or Cs-Glu pipettes ($P > 0.05$ Student’s $t$-test).

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**FIG. 8.** Inward currents induced by both SP (0.2 μM) and norepinephrine (NE; 5 μM) in the same neuron voltage clamped at −55 mV. Pipette was filled with Glu-K solution. Slice initially was perfused with standard external solution with TTX omitted. Note that as in the example shown in Fig. 2, transient inward currents, probably representing escaped spikes were seen. After adding TTX (10−6 M; indicated by arrow in bottom), response to NE but not to SP was abolished (the transient inward currents also were abolished). Note also that the effect of NE in this cell was accompanied by occurrence of spontaneous inward synaptic currents, which also were abolished by TTX. Also seen in response to both SP and NE was a decrease in the outward current evoked by depolarization. Representative current traces are shown (top) and their corresponding locations in the continuous recording (bottom) are indicated by letters (see Fig. 2A legend for protocol details). Dotted line indicates 0 current level.
P-INDUCED INWARD CURRENT IN AUDITORY EFFERENT NEURONS

Numerous brain stem structures have been found to be heavily innervated by SP-containing fibers (see Otsuka and Yoshioka 1993 for review). The SP projections to the auditory brain stem have not been described in detail until recently. In adult rats, SP-positive fibers and terminals form a curtain-like plexus dorsal, lateral, and rostral to the main nuclei of the superior olivary complex (SOC), while the VNTB appears to be the main defined nucleus of the SOC that shows a significant penetration by SP positive innervation (Wynne and Robertson 1997). This innervation probably does not arise from local structures because immunocytochemical evidence indicates that the SOC is devoid of SP-immunoreactive somata, and only weakly staining cells are seen in the cochlear nucleus and nuclei of the lateral lemniscus (Wynne and Robertson 1997). In situ hybridization results show an absence of mRNA for SP precursor in these regions as well (Wynne et al. 1995). There is evidence that the inferior colliculus (IC) expresses mRNA for SP precursor (Wynne et al. 1995) and contains SP-positive cells (Adams 1993), and a direct projection from the IC to MOCS neurons has been demonstrated in the rat (Vetter et al. 1993). Topographically, however, this projection does not appear to match the region of heavy mRNA expression for SP precursor in the IC. Recently a direct projection from auditory cortex to the VNTB has been demonstrated (Feliciano et al. 1995) that has a pattern of termination within the SOC that is strikingly similar to that described for SP-immunoreactive elements (Wynne and Robertson 1998), but as yet it is not known what transmitter this pathway uses or whether it terminates on MOCS neurons. Feliciano et al. (1995) have reported that corticofugal endings in the cochlear nucleus contain clear spherical synaptic vesicles consistent with an excitatory action, but they do not report the presence of dense-core vesicles that would be expected to be associated with a peptidergic mode of synaptic transmission. Thus the source of SP innervation of the VNTB remains unknown, and the possibility should be considered that the innervation may arise from nonauditory pathways.

Although the effects of MOCS activation on cochlear function are well documented, the real role in hearing of the olivocochlear pathway, remains to be satisfactorily resolved. Hence the precise functional significance of the results reported here await further investigation. MOCS neurons innervate the peripheral receptor organ, the cochlea, where they terminate on the outer hair cells (see Warr 1992 for review). Activation of the MOCS by electrical stimulation at high rates, suppresses auditory nerve fiber responses to sound through complex effects on the functioning of outer hair cells and their contribution to cochlear micromechanical behavior (e.g., Guinan and Gifford 1988a–c). It has been well established in both cat and guinea pig that MOCS can be driven by acoustic stimulation (Brown 1989; Liberman 1988; Liberman and Brown 1986; Robertson 1984; Robertson and Gummer 1985, 1988, and they therefore may provide a substrate for feedback regulation of the cochlea. Detectable changes in cochlear function after acoustic stimulation of the MOCS have been reported using a number of techniques, but the significance of these effects is unclear (Chery-Croze et al. 1993; Collet et al. 1990; Giraud et al. 1995; Liberman 1989; Mott et al. 1989). It also has been

**Nature and functional significance of SP pathways in auditory brain stem**

Because MOCS neurons in the VNTB are SP sensitive, it is obviously of interest to know the source of innervation of the VNTB that uses this neurotransmitter. Although numerous brain stem structures have been found to be heavily innervated by SP-containing fibers (see Otsuka and Yoshioka 1993 for review), the SP projections to the auditory brain stem have not been described in detail until recently. In adult rats, SP-positive fibers and terminals form a curtain-like plexus dorsal, lateral, and rostral to the main nuclei of the superior olivary complex (SOC), while the VNTB appears to be the main defined nucleus of the SOC that shows a significant penetration by SP positive innervation (Wynne and Robertson 1997). This innervation probably does not arise from local structures because immunocytochemical evidence indicates that the SOC is devoid of SP-immunoreactive somata, and only weakly staining cells are seen in the cochlear nucleus and nuclei of the lateral lemniscus (Wynne and Robertson 1997). In situ hybridization results show an absence of mRNA for SP precursor in these regions as well (Wynne et al. 1995). There is evidence that the inferior colliculus (IC) expresses mRNA for SP precursor (Wynne et al. 1995) and contains SP-positive cells (Adams 1993), and a direct projection from the IC to MOCS neurons has been demonstrated in the rat (Vetter et al. 1993). Topographically, however, this projection does not appear to match the region of heavy mRNA expression for SP precursor in the IC. Recently a direct projection from auditory cortex to the VNTB has been demonstrated (Feliciano et al. 1995) that has a pattern of termination within the SOC that is strikingly similar to that described for SP-immunoreactive elements (Wynne and Robertson 1998), but as yet it is not known what transmitter this pathway uses or whether it terminates on MOCS neurons. Feliciano et al. (1995) have reported that corticofugal endings in the cochlear nucleus contain clear spherical synaptic vesicles consistent with an excitatory action, but they do not report the presence of dense-core vesicles that would be expected to be associated with a peptidergic mode of synaptic transmission. Thus the source of SP innervation of the VNTB remains unknown, and the possibility should be considered that the innervation may arise from nonauditory pathways.

Although the effects of MOCS activation on cochlear function are well documented, the real role in hearing of the olivocochlear pathway, remains to be satisfactorily resolved. Hence the precise functional significance of the results reported here await further investigation. MOCS neurons innervate the peripheral receptor organ, the cochlea, where they terminate on the outer hair cells (see Warr 1992 for review). Activation of the MOCS by electrical stimulation at high rates, suppresses auditory nerve fiber responses to sound through complex effects on the functioning of outer hair cells and their contribution to cochlear micromechanical behavior (e.g., Guinan and Gifford 1988a–c). It has been well established in both cat and guinea pig that MOCS can be driven by acoustic stimulation (Brown 1989; Liberman 1988; Liberman and Brown 1986; Robertson 1984; Robertson and Gummer 1985, 1988, and they therefore may provide a substrate for feedback regulation of the cochlea. Detectable changes in cochlear function after acoustic stimulation of the MOCS have been reported using a number of techniques, but the significance of these effects is unclear (Chery-Croze et al. 1993; Collet et al. 1990; Giraud et al. 1995; Liberman 1989; Mott et al. 1989). It also has been

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reported that high levels of centrally driven activity in MOCS neurons can result in reversible hearing loss (Rajan 1989; Thompson 1993) and also that MOCS neurons can change their discharge characteristics as a result of sound conditioning (Kujawa et al. 1996). Recent interesting experiments in awake humans with vestibular nerve transection (de-efferented cochleas) suggest a role for the efferents in peripheral lateral inhibitory processes subserving selective attention (Scharf et al. 1997), implying a role for higher pathways in regulating peripheral auditory function through the MOCS neurons. If any of these roles for the MOCS neurons can be confirmed, a full understanding of how they regulate auditory processing will require the influence of neurotransmitters, such as SP and the neural pathways that employ them, to be taken into account. From previous microelectrode recordings in brain slices (Wang and Robertson 1997c, 1998) the activation of a SP-using pathway converging onto MOCS neurons would be expected to increase the overall output of this system and its effects on the auditory periphery. Under what precise circumstances such an influence might be exerted in the intact animal remains to be investigated.

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REFERENCES


P-INDUCED INWARD CURRENT IN AUDITORY EFFERENT NEURONS


