5-Hydroxytryptamine Responses in Immature Rat Rostral Ventrolateral Medulla Neurons In Vitro

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Hwang, L. L. and N. J. Dun. 5-Hydroxytryptamine responses in immature rat rostral ventrolateral medulla neurons in vitro. J. Neurophysiol. 80: 1033–1041, 1998. Whole cell patch recordings were made from rostral ventrolateral medulla (RVLM) neurons of brainstem slices from 8- to 12-day-old rats. By superfusion or pressure ejection to RVLM neurons, 5-hydroxytryptamine (5-HT) elicited three types of membrane potential changes: a slow hyperpolarization (5-HT1A), a slow depolarization (5-HT2D) and a biphasic response, which persisted in a tetrodotoxin (TTX, 0.3 μM)-containing solution. 5-HT1A were accompanied by a decrease of input resistance in the majority of responsive neurons. Hyperpolarization reduced and depolarization increased the 5-HT1A; the mean reversal potential was −92.3 mV in 3.1 mM and shifted to −69.3 mV in 7 mM [K+]o. Barium (Ba2+, 0.1 mM) but not tetraethylammonium (TEA, 10 mM) suppressed 5-HT1A. The 5-HT1A receptor agonist (±)-8-hydroxy-dipropylamino-tetralin (8-OH-DPAT; 5–50 μM) hyperpolarized RVLM neurons. The 5-HT1A antagonist pindolol-5-HT1A (PBD; 1–3 μM) and the 5-HT2/5-HT1 receptor antagonist spiperone (1–10 μM) suppressed 5-HT1A and the hyperpolarizing phase of biphasic responses; the 5-HT2 receptor antagonist ketanserin (3 μM) was without significant effect. 5-HT1A were associated with an increase or no apparent change of input resistance in RVLM neurons. Hyperpolarization of the membrane decreased or caused no apparent change in 5-HT2D. 5-HT2D were reduced in an elevated [K+]o (7.0 mM) solution and >60% in a low Na+(26 mM) solution and were not significantly changed in a low Cl−(6.7 mM) or Ca2+-free/high Mg2+(10.9 mM) solution. The 5-HT2 receptor agonist α-methyl-5-HT (50 μM) depolarized RVLM neurons, and the 5-HT2 antagonist ketanserin receptor (1–10 μM) attenuated the 5-HT2 and the depolarizing phase of biphasic responses, whereas the 5-HT1A receptor antagonist PBD (2 μM) was without effect. Inclusion of the hydrolysis resistant guanine nucleotide GDP-β-S in patch solution significantly reduced the 5-HT1A as well as the 5-HT2D. The present study shows that, in the immature rat RVLM neurons, 5-HT causes a slow hyperpolarization and depolarization probably by interacting with 5-HT1A and 5-HT2 receptors, which are G-proteins coupled receptors and/or increase of nonselective cation conductance.

INTRODUCTION

Neurons in the rostral ventrolateral medulla (RVLM), which subserve a number of physiological functions including cardiovascular, respiratory, and nociceptive, are a diverse group of neurons insofar as their transmitter phenotypes and target projections are concerned (Dampney 1994; Guyenet 1990). A reasonably well-characterized pathway involves a direct projection from a group of adrenergic neurons located in the RVLM to sympathetic preganglionic neurons in the intermediolateral column of the spinal cord. This group of RVLM neurons is thought to provide a major excitatory drive to sympathetic preganglionic neurons, which send their axons to the periphery and synapse with sympathetic postganglionic neurons. RVLM neurons in turn receive synaptic inputs, which are encoded with specific transmitters, from neurons within or outside the medulla (Dampney 1994).

Results from several studies support the idea that 5-hydroxytryptamine (5-HT) may be one of the putative transmitters involved in modulating the activity of a subset of RVLM neurons, consequently the cardiovascular dynamics. For example, RVLM neurons receive a moderately dense network of 5-HT-immunoreactive termini arising from dorsal raphe nuclei (Steinbusch 1981; Vertes and Kocsis 1994). Studies with extracellular recording techniques have shown that 5-HT or appropriate agonists acting via 5-HT1 and 5-HT2 receptors inhibited and excited bulbospinal sympathoexcitatory RVLM neurons (Wang and Lovick 1992a,b). Autoradiographic studies have demonstrated the presence of 5-HT1 and 5-HT2 receptors in the RVLM area (Pazos and Palacios 1985; Pazos et al. 1985). Last, 5-HT or selective agonists applied to the RVLM in vivo decreased or increased cardiovascular and respiratory activities by interacting with subtypes of 5-HT receptors (Gillis et al. 1989; Helke et al. 1992; Lovick 1989a,b; Mandal et al. 1990a,b; Nosjean and Guyenet 1991).

In a rat brain stem slice preparation, 5-HT has been found to hyperpolarize RVLM neurons (Lewis and Coote 1993), which may underlie the vasodepressant action of 5-HT in vivo. 5-HT microinjected into the RVLM has also been found to produce an excitatory action in vivo (Wang and Lovick 1992b). The cellular basis of the excitatory action of 5-HT on RVLM neurons has yet to be clarified. The present study was undertaken to reexamine the membrane effects of 5-HT on RVLM neurons in a coronal brain stem slice preparation similar to that described by Lewis and Coote (1993). The receptor subtypes and ionic mechanisms that may mediate the 5-HT responses were also explored.

METHODS

Coronal slices of 500 μm were prepared from immature (8- to 12-day-old) Sprague-Dawley rats (Zivic Miller, Zelienople, PA) anesthetized with ether and decapitated immediately (Lin et al. 1998). The brain was rapidly removed and placed in a Petri dish containing cold Krebs solution of the following composition (in mM): 127 NaCl, 1.9 KCl, 1.2 KH2PO4, 2.4 CaCl2, 1.3 MgCl2, 26 NaHCO3, and 10 glucose; the Krebs solution was gassed with 95% O2−5% CO2. The bulb was dissected and the pia mater carefully removed with a pair of fine forceps. The bulb was fixed onto an agar block with cyanoacrylic glue and sectioned with the use of a...
Vibratome. Slices containing the RVLM were incubated in oxygenated Krebs solution at room temperature (21 ± 1°C) for at least 1 h before the start of experiments. One slice was transferred to the recording chamber, held in place between two grids of fine nylon mesh, and superfused with oxygenated Krebs solution at a rate of 3–6 ml/min. All experiments were carried out in room temperature. In preparing Ca2+-free/high Mg2+ (10.9 mM) solution, CaCl2 was omitted and MgCl2 was increased accordingly. Elevated [K+]o solutions (7.0 mM) were prepared by substituting NaCl with KCl. Low [Na+]o solutions (26 mM) were made by substituting NaCl with Tris HCl titrated to pH 7.4, and NaCl was replaced with sodium isethionate in low [Cl−]o (6.7 mM) solutions. In preparing 10 mM tetrodylammonium (TEA)-containing Krebs solution, NaCl was replaced with TEA-Cl.

The whole cell patch recording technique was similar to that described earlier (Lin et al. 1998). Patch electrodes filled with a solution containing (in mM) 130 K+ gluconate, 1 MgCl2, 2 CaCl2, 4 ATP, 0.3 guanosine triphosphate (GTP), 10 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES) had a resistance of 2–5 MΩ; pH of the solution was adjusted to 7.2 with NaOH. Signals were recorded using an Axopatch 1C amplifier (Axon Instruments) in current-clamp mode and filtered at 2 kHz. The output was displayed on a Gould digital storage oscilloscope Model 1620 and a two-channel Gould chart recorder RS3200.

5-HT was applied either by pressure injection from a micropipette containing 5-HT (10 nM) placed close to the recording neuron and downstream from the inlet of perfusing Krebs solution with the use of a Picospritzer (General Valve) or by superfusion with a known concentration of 5-HT solution. The amount of 5-HT ejected from the micropipette was adjusted by varying the duration of ejection pulses while keeping the ejection pressure constant (40 psi). All other pharmacological agents were applied by superfusion in known concentrations.

The following compounds were used: 5-HT creatinine sulfate and guanosine 5’O-(2-thiophosphate) (GDP-β-S) from Sigma (St. Louis, MO), TEA chloride, tetrodotoxin (TTX), spiperone HCl, (±)-8-hydroxy-dipropylamino-tetralin (8-OH-DPAT), pin- dibind-5-HT1A (PBD), ketanserin, and α-methyl-5-HT from Research Biochemicals (Natuck, MA). Data are expressed as means ± SE and analyzed statistically by means of the Student’s t-test with a significance at P < 0.05.

**RESULTS**

**RVLM neurons**

Whole cell patch recordings were made from neurons in the RVLM area. The RVLM was recognized in the slice as an area ventral to the compact region of nucleus ambiguus, which appeared as a slightly dark area in a freshly prepared medulla slice, and lateral to the paragigantocellular nucleus at the level rostral to area postrema (Lewis and Coote 1993; Lin et al. 1998). While the general location, RVLM neurons were not further characterized with respect to their transmitter phenotype or physiological function.

RVLM neurons had a mean resting membrane potential, spike height, and input resistance of −57 ± 0.9 (SE) mV, 70.4 ± 1.5 mV, and 533.6 ± 37.7 MΩ (n = 74). As previously reported (Lewis and Coote 1993; Lin et al. 1998), a population of RVLM neurons discharges spontaneously. Because of membrane oscillations and discharges in these cells, it was difficult to measure accurately the 5-HT responses under these conditions. As a result, TTX (0.3 μM) was included in the Krebs solution to block spontaneous spike discharges in these neurons. Under these conditions, 5-HT caused a depolarization in 11 neurons (9.0 ± 1.3 mV), a hyperpolarization (9.8 ± 3.6 mV) in 5 neurons, and no detectable response in 4 of the spontaneously firing RVLM neurons. When compared with a similar sample size of silent neurons, 5-HT depolarized 14 neurons (7.5 ± 1.0 mV), hyperpolarized 7 neurons (7.5 ± 0.9 mV), and had no detectable effect in 4 neurons. Because 5-HT responses in spontaneously firing and quiescent neurons were comparable, the data were pooled from these two types of RVLM neurons.

**5-HT responses in RVLM neurons**

Pressure applications of 5-HT to 175 RVLM neurons resulted in a slow 5-HTH or slow 5-HTD in 51 and 58 neurons or a biphasic response with hyperpolarization followed by depolarization in 16 neurons; a response was not detected in the remaining neurons. Representative responses are shown in Fig. 1. The onset of 5-HT in induced by a puff was usually <3 s as compared with 5 s in the case of 5-HT. The amplitude and duration of 5-HTH and 5-HTD were related to the duration of puff in a given neuron (Fig. 1). Further, 5-HTH, 5-HTD, or biphasic responses persisted in a Ca2+-free/high Mg2+ solution, and in a solution containing TTX (0.3 μM) in all 14 neurons tested.

Similar to puff applications, superfusion of 5-HT (10–50 μM) caused a slow depolarization in 23/44 neurons and a slow hyperpolarization in 12/44 neurons. Figure 2 shows the concentration-effect relations for 5-HTH and 5-HTD. A small (1–3 mV) and transient hyperpolarization that preceded a depolarization was noted in three neurons, and a small depolarization following wash out of 5-HT was detected in four neurons in which 5-HT caused a hyperpolarization only (Fig. 4B). Although the possibility that pressure ejection may activate mainly somatic receptors and superfusion may activate the somatic and dendritic receptors cannot be excluded, the response and direction of membrane resistance change in a given neuron was the same whether applied by bath or by pressure ejection.

**5-HT hyperpolarizations**

At the membrane potential of −60 mV, 5-HTH were concentration dependent and ranged from a few to >10 mV in different cells (Fig. 2A). 5-HTH in 9/13 neurons (69%) were associated with a decrease of input resistance ranging from 10 to 41% (22.1 ± 3.6%; n = 9), measured at the time when the potential shift was offset by passing current through the recording electrode (Fig. 7C). In the other four neurons (31%), the responses were not accompanied by a detectable change in input resistance. In spite of the differences in membrane resistance change, the mean amplitude of 5-HTH in these two groups of neurons was comparable, i.e., 8.1 ± 1.4 versus 8.4 ± 1.7 mV.

5-HTH became larger upon depolarization and smaller upon hyperpolarization (Fig. 3). The extrapolated mean reversal potential was −92.3 ± 3.3 mV in 3.1 mM [K+], which is close to the K+ equilibrium potential (E<sub>K</sub>) of −94 mV calculated from the Nernst equation at 20°C. The mean reversal potential shifted to −69.3 ± 2.3 mV (n = 3) in 7 mM [K+], solution, as shown in Fig. 3.
Superfusing the slices with a Krebs solution containing Ba\(^{2+}\) (0.1 mM) significantly reduced the 5-HT\(_h\); the mean amplitude was 7.3 ± 1.4 mV in Krebs solution and 1.3 ± 0.9 mV in Ba\(^{2+}\) solution (n = 3; P = 0.027). 5-HT\(_h\) were not significantly affected by TEA (10 mM) in three neurons tested (P > 0.05).

5-HT\(_{1A}\)–like receptors

The selective 5-HT\(_{1A}\) agonist 8-OH-DPAT (5–50 μM) (Gozlan et al. 1983) produced a slow hyperpolarization comparable in amplitude with that caused by 5-HT (n = 7). 8-OH-DPAT hyperpolarizations had a duration of 30–50 min, which was noticeably longer than that of 5-HT\(_h\) (Fig. 4A). Further, 5-HT was unable to evoke a detectable response when applied during 8-OH-DPAT–induced hyperpolarization (n = 3) or elicited a smaller response when applied immediately after the membrane potential had recovered from 8-OH-DPAT–induced hyperpolarization (Fig. 4A).

Pretreating the slices with the selective 5-HT\(_{1A}\) antagonist PBD (Liau et al. 1991) concentration dependently and reversibly suppressed the 5-HT\(_h\) (n = 5; Fig. 4B) and the hyperpolarizing phase of the biphasic responses (n = 3; Fig. 7B). At the concentrations of 1, 2, and 3 μM, PBD suppressed 5-HT\(_h\) by 84.0 ± 10.3% (n = 5), 92.0 ± 8.0% (n = 5), and 98.0 ± 2.0% (n = 5). The 5-HT\(_2\)/5-HT\(_{1}\) receptor antagonist spiperone (1–10 μM) (Peroutka and Snyder 1979) partially or completely suppressed the 5-HT\(_h\) in three cells tested; the onset and recovery of the blocking action of spiperone was slow (Fig. 4C). The 5-HT\(_2\) receptor antagonist ketanserin (3 μM) (Van Nueten et al. 1981) had no significant effect on 5-HT\(_h\) in three neurons (96.7 ± 3.3%).

FIG. 1. Dose-dependent 5-hydroxytryptamine (5-HT) responses in rostral ventrolateral medulla (RVLM) neurons. In all cases, 5-HT was applied to RVLM neurons by pressure ejections as indicated by arrows. Amplitude and duration of hyperpolarizing, depolarizing, and biphasic responses were related to the ejection pulse durations as indicated. Downward deflections are hyperpolarizing electrotonic potentials used to monitor input resistance. A: 5-HT elicited a slow hyperpolarization. B: 5-HT elicited a slow depolarization upon reaching the threshold fired several action potentials. C: 5-HT evoked a biphasic response. Low-amplitude excitatory and/or inhibitory postsynaptic potentials, which appear as synaptic noises, occurred in A–C. The resting membrane potentials of neurons A–C were −60, −55, and −61 mV.

FIG. 2. Dose-response relations of 5-HT depolarizations and hyperpolarizations in rat RVLM neurons. 5-HT was applied by bath in all cases. Data points represent means ± SE of 4–9 neurons.
5-HT depolarizations

At the resting potential of about −60 mV, 5-HT_2D_ ranged from a few to >10 mV (Fig. 2B). An increase (13/29 neurons, 28.4 ± 5.2%; Fig. 6C) or no apparent change (16/29) in input resistance was noted during the course of 5-HT_2D_. After the removal of 5-HT from the perfusing solution, the membrane potential recovered slowly.

Two types of 5-HT_2D_ that differed in their voltage-response relations were noted. In the first type, 5-HT_2D_ were accompanied by an increase or no apparent change of input resistance, and the responses became larger upon depolarization and smaller upon hyperpolarization in 5 of 13 neurons tested. In the second type (n = 8), the amplitude of 5-HT_2D_ was relatively independent of membrane potentials. Both types of 5-HT_2D_ were not significantly changed in a Ca^{2+}-free/high Mg^{2+} solution (12.8 ± 1.6 mV vs. 13.9 ± 2.2 mV; n = 5) nor in a low Cl⁻ (6.7 mM) solution (15.4 ± 4.7 mV vs. 14.1 ± 4.8 mV; n = 5). Increased [K⁺]₀ from 3.1 to 7.0 mM caused a small decrease in 5-HT_2D_ (15.8 ± 5.6 mV vs. 11.0 ± 4.7 mV; n = 3; Fig. 5A). In a low Na⁺ (26 mM) solution, the 5-HT_2D_ were significantly reduced (11.5 ± 5.6 mV vs. 4.6 ± 1.9 mV; n = 5; Fig. 5B).

5-HT_2-like receptors

The 5-HT_2 receptor agonist α-methyl-5-HT (50 μM) (Richardson et al. 1985) mimicked the slow depolarization induced by 5-HT in all five neurons tested (11.6 ± 4.2 mV vs. 12.0 ± 3 mV; Fig. 6A). The 5-HT_2 receptor blocker ketanserin suppressed 5-HT_2D_ (Fig. 6B). At the concentrations of 1, 5, and 10 μM, ketanserin suppressed the 5-HT_2D_ by 47.7 ± 13.2% (n = 8), 73.3 ± 9.0% (n = 8), and 87.4 ± 9.0% (n = 5); it also eliminated the depolarizing phase of the biphasic responses (Fig. 7A). The blocking effect of ketanserin was long lasting; 5-HT_2D_ did not fully recover even after a prolonged (>2 h) wash. To minimize the potential for tachyphylaxis, responses evoked by the first application of 5-HT in the presence and absence of ketanserin (2 μM) were compared. 5-HT_2D_ occurred in 5/19 neurons (26%) and 23/44 neurons (52%) in the presence and absence of ketanserin, respectively. The mean amplitude of 5-HT_2D_ obtained from five neurons in the presence of ketanserin was 4.9 ± 1.2 mV, which was smaller than the mean amplitude of 11.0 ± 1.5 mV (n = 23; P = 0.07) obtained from the group of neurons not pretreated with ketanserin. 5-HT_2D_ were not significantly affected by the 5-HT_1 antagonist PBD (2 μM) in three neurons tested (11 ± 3.8 mV vs. 10.7 ± 3.2 mV).

Biphasic responses

Application of 5-HT by pressure ejection produced a biphasic response, which was composed of an initial hyperpolarization followed by a slow depolarization of varying amplitude, in 16/175 neurons. Ketanserin (1 μM) preferentially suppressed the depolarizing phase of the biphasic responses, consequently, enhancing the initial hyperpolarization (n = 3; Fig. 7A). PBD (1 μM) selectively eliminated the hyperpolarizing phase of the biphasic response, thereby augmenting the depolarizing phase (Fig. 7B, n = 3). Both phases were abolished by spiperone (10 μM; Fig. 7A). Bath application of 5-HT also caused a biphasic response (7/44) as shown in Fig. 7C. Ketanserin (2 μM) suppressed the depolarizing and increased the hyperpolarizing phase in three neurons tested.

Involvement of GTP-binding proteins in 5-HT responses

In this series of experiments, neurons were recorded with patch electrodes filled with a solution containing the hydrolysis-resistant guanine nucleotide GDP-β-S (1 mM). GDP-β-S binds to G-proteins and inhibits the binding of GTP, thereby blocking the GTP-dependent activation of G-proteins. Responses to the first application of 5-HT shortly after the rupture of recording neuron membrane were compared...
FIG. 4. 5-HT hyperpolarizations mimicked by (+)-8-hydroxy-dipropylamino-tetralin (8-OH-DPAT) and antagonized by pindobind-5-HT₁A (PBD) or spiperone in RVLM neurons. A: superfusion of 5-HT (10 μM) elicited a slow hyperpolarization with a peak amplitude of 8 mV. Superfusion of 8-OH-DPAT (30 μM) to the same neuron caused a longer lasting slow hyperpolarization of about the same amplitude. After a period of 40 min wash, the membrane potential had returned to the control level, and a 2nd application of 5-HT induced a smaller hyperpolarization of ~5 mV. B: pretreating the slice with PBD (1 μM) significantly reduced the hyperpolarization from 8 to 3 mV induced by the 2nd application of 5-HT, and the blocking effect of PBD was reversible. C: spiperone slowly antagonized the hyperpolarizations evoked by pressure ejection of 5-HT from 5 mV to ~1 mV. Numbers in minute indicate the time after spiperone application or wash. Recordings in A-C were from 3 different RVLM neurons that had resting membrane potentials of -56, -56, and -62 mV.

with that obtained with the second application, which took place 30 min later. Both 5-HTᵰ and 5-HTᵱ evoked by the second application of 5-HT were smaller (24.5 ± 9.2%, n = 5 and 10.8 ± 5.8%, n = 3) than that of the first application (Fig. 8, A and B).

Because GDP-β-S was a trilithium (Li⁺) salt, experiments were performed to evaluate the possibility that Li⁺ itself may affect 5-HT responses. In these experiments, LiCl (3 mM) was added to the patch solution and introduced into the recorded neurons. Li had little effect on the amplitude of 5-HTᵰ in five neurons tested. Last, hyperpolarizations mediated by glycine receptors, which are ligand gated, were evaluated as a negative control. In four neurons, the mean amplitude of glycine hyperpolarizations obtained shortly after and 30 min after rupturing the membrane was 8.5 ± 2.1 mV and 8.2 ± 2.3 mV.

DISCUSSION

The present study provides the first account of a depolarizing, hyperpolarizing, or a biphasic response by 5-HT in immature rat RVLM neurons of brain stem slices. Because the responses were not significantly changed in a TTX-containing or a Ca²⁺-free solution, 5-HT acted directly on RVLM neurons from which the recordings were made. Our result differs from an earlier study in which 5-HT was found
Fig. 5. Ion substitutions on 5-HT depolarizations in RVLM neurons. 5-HT (50 μM) was applied by bath for a period indicated by a bar. A: 5-HT depolarization was reduced by ~20% in 7.0 mM [K⁺]₀ solution. B: perfusing the slice with a low Na⁺ (26 mM) solution reduced the 5-HT depolarization to <30% of control response. Recordings were obtained from 2 different RVLM neurons that had resting potentials of -67 and -58 mV.

Fig. 6. α-Methyl-5-HT mimicked and ketanserin antagonized 5-HT depolarizations in RVLM neurons. A: 5-HT elicited a slow depolarization with a burst of discharges at the peak; α-methyl-5-HT (50 μM) mimicked the response. B: the depolarization evoked by bath application of 5-HT was markedly reduced by superfusion of ketanserin (1 μM); intense discharge was noted on the plateau phase. C: 5-HT caused a slow depolarization associated with an increase in membrane resistance (~30%), which persisted when the potential shift was offset by returning the membrane potential to the resting level. Recordings in A–C were from different RVLM neurons that had resting potentials of -60, -63, and -57 mV, respectively.

5-HT₁₅-like receptor-mediated hyperpolarization

Pharmacological studies suggest that the 5-HT₁₅ in RVLM neurons is likely to be mediated by 5-HT₁₅ or closely related receptors. First, the selective 5-HT₁₅ receptor agonist 8-OH-DPAT produced a hyperpolarization with characteristics similar to that of 5-HT₁₅. Second, 5-HT failed to elicit a hyperpolarization during 8-OH-DPAT–induced hyperpolarization or elicited a much smaller response during the recovery phase, suggesting that agonist-induced receptor desensitization may have occurred (Harrington et al. 1994). Third, the antagonism of 5-HT₁₅ by the 5-HT₁₂ receptor antagonist spiperone, but not by the 5-HT₂ receptor antagonist ketanserin, argues against a major contribution by 5-HT₂ receptors. Last, the selective 5-HT₁₅ receptor antagonist PBD eliminated the 5-HT₁₅. These findings in conjunction with the earlier observations of the detection of 5-HT₁₅–binding sites (Thor et al. 1990) and inhibition of the activity of RVLM neurons by activation of 5-HT₁₅ receptors in vivo (Wang and Lovick 1992a) support an inhibitory role of 5-HT₁₅ receptors in the RVLM.

5-HT₁₅: K⁺ conductance increase

Insofar as the ionic mechanism is concerned, the findings that 5-HT₁₅ were associated with a decrease of membrane resistance, reduced by membrane hyperpolarization and nulled near the E₉ are consistent with the idea that 5-HT hyperpolarizes RVLM neurons by opening K⁺ channels. This is further supported by the observation that the extrapo-
lated reversal potential shifted to a positive level in an elevated \( [K^+]_o \) solution as predicted by the Nernst equation for \( K^+ \) ions.

The specific type(s) of \( K^+ \) channels coupled to the 5-HT\(_{1A}\) receptor in the RVLM neurons remains to be fully delineated. The finding that 5-HT\(_H\) were not affected by a relatively high concentration of TEA but markedly reduced by a low concentration of Ba\(^{2+}\), which is known to block an inwardly rectifying \( K^+ \) current in various central neurons (Bobker and Williams 1995; Uchimura et al. 1989; Williams et al. 1988) suggests that 5-HT may hyperpolarize RVLM neurons by activating an inwardly rectifying \( K^+ \) current. In this respect, the \( K^+ \) conductance affected by 5-HT has been identified as an inwardly rectifying current in hippocampal CA1 and CA3 neurons (Andrade and Nicoll 1987; Colino and Halliwell 1987; Okuhara and Beck 1994), rat dorsal raphe neurons (Penington et al. 1993; Williams et al. 1988), and guinea pig nucleus propositus hypoglossi (Bobker and Williams 1995).

First, the observation that intracellular dialysis with GDP-\( \beta \)-S markedly reduced the 5-HT\(_H\) is consistent with the involvement of G-proteins in coupling 5-HT\(_{1A}\) receptors to

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**FIG. 7.** Antagonism of 5-HT biphasic responses by subtypes of 5-HT receptor antagonists. A: ketanserin (1 \( \mu \)M) preferentially antagonized the depolarizing phase, and spiperone (10 \( \mu \)M) abolished both depolarizing and hyperpolarizing phases. B: PBD (1 \( \mu \)M) preferentially suppressed the hyperpolarizing phase and enhanced the depolarizing phase in this neuron. C: ketanserin preferentially blocked the depolarizing phase and enhanced the hyperpolarizing phase in this neuron. There was a decrease of input resistance during 5-HT hyperpolarization, which persisted when the potential shift was offset by returning the membrane potential to the resting level. The resting membrane potentials of neurons A—C were −58, −57, and −61 mV and indicated by dotted lines.

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**FIG. 8.** Suppression of 5-HT response by intracellular dialysis of GDP-\( \beta \)-S (1 mM) in RVLM neurons. Neurons were recorded with patch electrodes containing GDP-\( \beta \)-S solution. 5-HT (50 \( \mu \)M) was applied by superfusion (solid bars) in all experiments. A and B: hyperpolarization or depolarization induced by 2nd application of 5-HT was greatly reduced as compared with 1st application of 5-HT shortly after the rupture of the recorded neurons.
the opening of K⁺ channels, as has been proposed in other central neurons (Andrade et al. 1986; Okuhara and Beck 1994; Penington et al. 1993; Williams et al. 1988).

5-HT₂-like receptor-mediated depolarization

Although a depolarization by 5-HT was not reported in RVLM neurons of brain stem slice preparations (Lewis and Coote 1993), the finding that iontophoresis of the 5-HT₂ receptor agonist α-methyl-5-HT increased the firing rate of RVLM neurons in situ could be interpreted as an excitation of these neurons (Wang and Lovick 1992b). Our result clearly shows that 5-HT depolarized or caused a biphasic response in a population of RVLM neurons.

The type(s) of receptors mediating the 5-HT_D were analyzed pharmacologically. First, the 5-HT₂ agonist α-methyl-5-HT mimicked the 5-HT_D. Second, 5-HT antagonists known to block the 5-HT₂ receptor including spiperone and ketanserin, were effective in suppressing the 5-HT_D. In addition, others have reported the presence of 5-HT₂-like receptors in RVLM areas by immunohistochemical techniques (Pazos et al. 1985). Taken together, these results support the contention that 5-HT depolarizes RVLM neurons by interacting with 5-HT₂-like receptors.

Ionic basis of 5-HT_D

Two types of voltage-response relations with respect to 5-HT_D are noted in RVLM neurons. In the first type, 5-HT_D were accompanied by an increase or no apparent change of input resistance, and the responses were reduced upon membrane hyperpolarization or in a high [K⁺]o solution. These findings indicate that 5-HT depolarized RVLM neurons by primarily closing K⁺ conductance. Some of the RVLM neurons showed no apparent change in input resistance during 5-HT_D, indicating that other ions in addition to K⁺ ions may be involved. In the second type, 5-HT_D were relatively independent of membrane potentials, indicating the possible involvement of multiple conductance changes. Iontophoretic experiments show that 5-HT_D were reduced by low [Na⁺]o solution in both types of neurons, suggesting that 5-HT may increase Na⁺ conductance. The two types of voltage-response relation observed in 5-HT_D may be explained by the relative percent of K⁺ conductance decrease and Na⁺ conductance increase by 5-HT in a single RVLM neuron. It is not known whether a single class of 5-HT₂ receptors in the brain stem neurons may couple to different ion channels or the latter are coupled to subtypes of 5-HT₂ receptors. That 5-HT_D may result from activation or inactivation of different ion channels has been reported in a variety of central neurons. For example, 5-HT by closing primarily K⁺ channels depolarizes somatosensory cortex neurons (Davies et al. 1987), prefrontal cortex neurons (Araneda and Andrade 1991), and spinal motoneurons (Wang and Dun 1990). On the other hand, Bobker and Williams (1989) have demonstrated that 5-HT augmented a time- and voltage-dependent nonselective cation current, i_h, in rat nucleus propositus hypoglossi. Similar responses had been reported in lateral geniculate neurons of the guinea pig and cat (Pape and McCormick 1989) and rat spinal motoneurons (Takahashi and Berger 1990).

Last, 5-HT_D were much smaller in neurons intracellularly dialyzed with GDP-β-S, suggesting the 5-HT₂ receptors in question are G-protein coupled, as has been proposed in other central neurons (Boess and Martin 1994).

Biphasic response

5-HT elicited a biphasic response, which persisted in a TTX-containing Krebs solution, in a population of RVLM neurons, indicating the activation of two subtypes of 5-HT receptors. The coexistence of two subtypes of 5-HT receptors on a single RVLM neuron is further indicated by the observation that the biphasic response was blocked by a combination of the putative 5-HT₁A receptor antagonist PBD and 5-HT₂ receptor antagonist ketanserin. 5-HT interacting with subtypes of 5-HT receptors has been shown to depolarize and hyperpolarize a variety of neurons in the brain (Andrade and Nicoll 1987; Araneda and Andrade 1991; Davies et al. 1987). An interesting question that remains to be addressed is whether subtypes of 5-HT receptors may be distributed topographically in a RVLM neuron.

Physiological significance

RVLM neurons receive a moderately dense innervation of 5-HT-immunoreactive fibers (Steinbusch 1981). Iontophoretic application of 5-HT or agonists to RVLM neurons in situ increased and/or decreased blood pressure and/or heart rate (Gillis et al. 1989; Laubie et al. 1989; Lovick 1989a,b; Wang and Lovick 1992a,b). Our result clarifies the possible mechanism with respect to the excitatory and inhibitory action of 5-HT on RVLM neurons. It may be surmised that 5-HT released from serotonergic terminals interacts with subtypes of receptors to either depolarize or hyperpolarize RVLM neurons. Similar to other G-protein coupled receptors, the time course of 5-HT_D and 5-HT_D is relatively slow as compared with glutamate receptor-mediated responses. This may provide the RVLM neuron a means to adjust the membrane excitability over a period of seconds to minutes, rather than an impulsive change of membrane potentials brought on by glutamatergic transmission.

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