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A Slow Excitatory Postsynaptic Potential Mediated by 5-HT_2 Receptors in Nucleus Prepositus Hypoglossi

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Intracellular recordings were made from neurons of the nucleus prepositus hypoglossi in slices of guinea pig medulla. 5-HT (serotonin) caused a hyperpolarization followed by a late depolarization. The hyperpolarization was mediated by 5-HT_{1A} receptor activation and could be selectively blocked by pindobind-5HT_{1A} (PBD). 5-HT then caused a depolarization only. A selective 5-HT_2 agonist, (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), also caused a depolarization. Ketanserin and spiperone, 5-HT_2 antagonists, blocked the depolarization due to both 5-HT and DOI.

Focal electrical stimulation caused an IPSP mediated by 5-HT acting upon 5-HT_{1A} receptors and a slow EPSP (s-EPSP). PBD blocked the IPSP, leaving an isolated s-EPSP. Both spiperone and ketanserin antagonized the s-EPSP, while DOI occluded it. The s-EPSP was from 2 to 10 mV in amplitude and 35–50 sec in duration, and showed voltage dependence consistent with a decrease in potassium conductance. Both the IPSP and the s-EPSP were presynaptically inhibited by the 5-HT_{1D} agonist sumatriptan. These data indicate that the s-EPSP is mediated by 5-HT acting upon 5-HT_2 receptors. This represents strong support for the role of 5-HT as an excitatory neurotransmitter in the CNS. Further, it demonstrates that synaptic release of 5-HT can mediate opposing effects on the membrane potential of a single cell.

[Key words: prepositus hypoglossi, slow EPSP, 5-HT_2 receptor, serotonin, sumatriptan, pindobind-5HT_{1A}, (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)]

5-HT (serotonin) has been reported to cause neuronal excitations through activation of 5-HT_2, 5-HT_3, 5-HT_4, and still unidentified receptors (Gerschenfeld and Paupardin-Tritsch, 1974; Bobker and Williams, 1990a; Andrade and Chaput, 1991; Anwyl, 1992). Among the best described of these is the depolarization mediated by the 5-HT_2 receptor. This response has been observed in neurons of rat facial motor nucleus (VanderMaelen and Aghajanian, 1980), association cortex (Araneda and Andrade, 1991), nucleus accumbens (North and Uchimura, 1989), and guinea pig somatosensory cortex (Davies et al., 1987). A slow excitatory postsynaptic potential (s-EPSP) resulting from 5-HT_2 receptor activation has been reported in rat spinal motoneurons (Wang and Dun, 1990) and in the guinea-pig nucleus prepositus hypoglossi (PH), although it has been only briefly characterized (Bobker and Williams, 1991). A more detailed analysis of this s-EPSP in the PH is the subject of this study.

The 5-HT_2 receptor is a member of a family of proteins that includes the 5-HT_{1C}, α1 adrenergic, M_1 muscarinic, and other receptors (Conn and Sanders-Bush, 1984; Berridge, 1993) and has been implicated in hallucinogenesis (Glennon, 1984), vasoconstriction (Golino et al., 1991), and sleep regulation (Stutzmann et al., 1992). These receptors couple to phosphatidylinositol turnover via a G-protein. The resulting second messengers are diacylglycerol-activated protein kinase C and inositol(1,4,5)-trisphosphate. The latter causes a rise in intracellular free calcium, which may be responsible for the change in ionic conductance mediated by these receptors. Most electrophysiological studies have implicated a potassium conductance decrease as underlying the 5-HT_2–evoked depolarization (VanderMaelen and Aghajanian, 1980; Davies et al., 1987; North and Uchimura, 1989; Wang and Dun, 1990; Araneda and Andrade, 1991). This excitation has the slow onset and long time course expected of a second messenger–mediated event.

The goals of the present study were (1) to demonstrate that the s-EPSP observed in PH neurons was mediated by the 5-HT_2 receptor, (2) to characterize the time course of the s-EPSP, and (3) to determine if the s-EPSP was subject to autoreceptor inhibition.

Materials and Methods

Intracellular recordings. The methods used were identical to those published previously (Bobker and Williams, 1991). Male guinea pigs (200–350 gm) were anesthetized with halothane and killed. Slices of brainstem (thickness of 300 μm) containing the PH were cut in the horizontal plane in a vibratome, submerged in a tissue bath (volume of 0.5 ml), and superfused with physiological saline (1.5 ml/min) at 35°C. The content of the superfusate was (mM) NaCl, 126; KCl, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $CaCl_2$, 2.4; glucose, 11; and $NaHCO_3$, 24, saturated with 95% O_2, 5% CO_2. The nucleus PH was located by its position adjacent to the fourth ventricle in the medulla (Paxinos and Watson, 1982). Microelectrodes were filled with 2 M KCl and were from 40 to 80 MΩ in resistance. Synaptic potentials were evoked using focal electrical stimulation (constant current stimulator at 0.5–5 mA, 0.5–1 msec, single stimulus delivered every 60–90 sec) with bipolar tungsten electrodes placed in the slice approximately 500–1000 μm apart and within 500 μm of the recording electrode. Supramaximal stimulus was used for all studies.

Voltage-clamp studies were performed using discontinuous single-electrode voltage clamp with a switching frequency of 3–5 kHz. The headstage was monitored continuously for adequacy of voltage clamping.

Drug application. Drugs were applied in known concentrations by changing the perfusion solution to one that differed only in its content of drug. Drug used were 5-HT creatinine sulfate, (+)cocaine HCl, prazosin (Sigma Chemical Co.); pindobind-5HT_{1A} (PBD), (+)cyanopindolol, 1-(2-methoxyphenyl)-4-[4-(2-phthalimmido)butyl]piperazine HBr

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(NAN-190), (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), spiperone, ketanserin, (+)sulpiride (Research Biochemicals Inc.); and sumatriptan (gift from Glaxo).

Data analysis. Measurement of current and voltage was done with an Axoclamp-2A amplifier and recorded either directly onto a chart recorder or onto CHART or SCOPE data acquisition programs (AD Instruments, Castle Hill, Australia). For all measurements, three consecutive synaptic potentials were averaged. Determination of the s-EPSP time-to-peak (TTP) and duration was done by measuring the time from stimulation artifact to peak amplitude and then return to baseline potential, respectively. Analysis of time constants for the isolated s-EPSP was done using a double exponential fit program (AXOGRAPH, Axon Instruments). Curve fitting was done between the start of the rising phase of the s-EPSP and the return to baseline. A single exponential, fit between peak amplitude and return to baseline, was used to determine the time constant of decay for the s-EPSP in control. Inhibitory postsynaptic potential (IPSP) duration was measured from stimulus artifact to return to baseline; time constants were not determined due to the presence of the s-EPSP, but have been reported previously where the s-EPSP was blocked (Bobker and Williams, 1991). Reversal potentials were determined using least-squares regression analysis. Cell firing rates were determined by taking a 1–3 sec sample at baseline and during the peak effect of the s-EPSP. The statistical significance of the difference between mean values of samples was determined using Student's t test for paired means. Where differences between means are given, P was less then 0.05. All mean values are expressed as the standard error of the mean.

Results

These results are based on recordings made from 50 neurons. The membrane properties of these cells have been reported previously. Briefly, type I PH neurons have a fast action potential (about 0.5 msec), are spontaneously active (firing frequencies from 1 to 20 Hz), respond to 5-HT in a biphasic manner (hyperpolarization followed by depolarization), and are found within 500 μm of the wall of the fourth ventricle. Two other cell types have been observed. Type II neurons have a broader action potential, are hyperpolarized by 5-HT, and are located in the most rostral region of the nucleus (Bobker and Williams, 1990b). Type III neurons are also spontaneously active with a fast action potential, are depolarized by 5-HT, and are located from 300 to 1000 μm from the ventricle. The 5-HT receptor in the latter group of neurons is distinct from described 5-HT receptors and depolarized neurons by activating a hyperpolarization-activated current (h-current; see Bobker and Williams, 1989a). Therefore, this study included type I cells only, which could generally be selected on the basis of their location close to the ventricle.

5-HT$_{1A}$ and 5-HT$_2$ receptors mediate opposing actions

5-HT had a biphasic effect on membrane potential. From a holding potential of −65 to −70 mV, bath perfusion of 5-HT (30 μM) caused a 10–25 mV hyperpolarization, sometimes followed by a depolarizing "sag" in the response. With washout, most cells (approximately 80%) demonstrated a 2–5 mV depolarizing overshoot of the baseline potential (Fig. 1). This biphasic effect was seen at lower concentrations as well, although the lowest doses (1–3 μM) caused a hyperpolarization with no discernible depolarization. A previous report has characterized the 5-HT–induced hyperpolarization as being due to activation of 5-HT$_{1A}$ receptors (Bobker and Williams, 1990b). To isolate the 5-HT–induced depolarization, several antagonists at the 5-HT$_{1A}$ receptor were tested. Spiperone (1 μM) and NAN-190 (100–300 nM) inhibited both the hyperpolarization and the depolarization. PBD, a more selective 5-HT$_{1A}$ antagonist (Liau et al., 1991), inhibited the 5-HT–induced hyperpolarization without significantly affecting the depolarization. Following superfusion with PBD (1 μM), 5-HT caused a depolarization in five

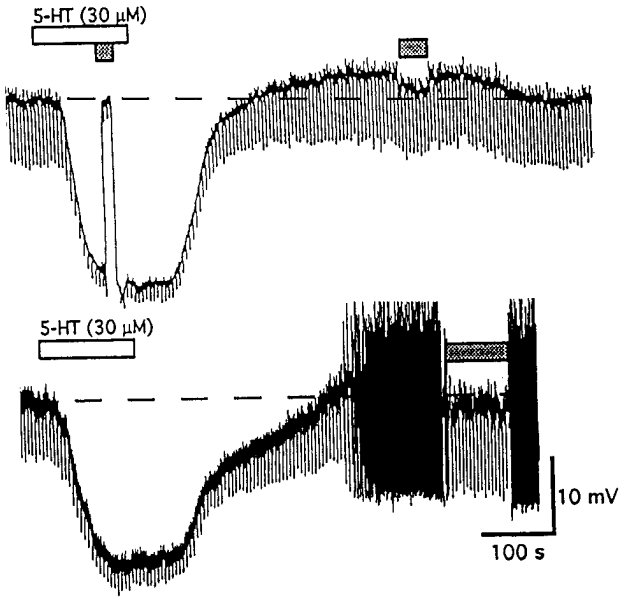

Figure 1. 5-HT had a biphasic effect on membrane potential. *Upper trace*, Superfusion of 5-HT (*open bar*) caused a membrane hyperpolarization (holding potential was −70 mV, *dashed line*); with washout, the potential overshoots with a late depolarization. The brief downward deflections are electrotonic potentials in response to hyperpolarizing current pulses (400 msec). Current was passed (*shaded bars*) to measure the change in input resistance with manual voltage clamp. This demonstrates a decrease in membrane resistance during the hyperpolarization, but no apparent change during the depolarization. *Lower trace*, In another cell the late depolarization caused an increase in membrane resistance (approximately 70%). The truncated spikes are action potentials caused by the depolarization.

of seven cells, while two cells still had a small hyperpolarization. Associated with the depolarization was an increase in membrane resistance (70–80%) in four of seven cells and no change in three cells (Fig. 1). The depolarization reached equilibrium in 1–3 min, did not desensitize with applications for up to 10 min, and reversed completely within 10 min of washout. In comparison, the 5-HT–induced hyperpolarization reached equilibrium in 0.5–2 min and washed out within 5 min.

Concentration–response curves for 5-HT were performed in the presence of cocaine (10 μM; due to an avid reuptake system in this preparation; see Bobker and Williams, 1991) and PBD (1 μM). The depolarization, which was from 2 to 10 mV, frequently caused the cell to begin firing action potentials. Concentration–response curves were therefore expressed as the amount of current applied to maintain a steady potential, that is, manual voltage clamp. The onset of effect was at 10 nM and peaked at 300 nM. The EC_{50} for 5-HT was 23 ± 1 nM, with a maximum effect of 71 ± 20 pA of inward current (n = 4; Fig. 2*A*).

DOI, a high-affinity agonist at the 5-HT$_2$ receptor (Glennon et al., 1984), caused a depolarization only. The TTP effect was longer than for 5-HT, requiring about 3–5 min, and washout occurred over 20–30 min. The response to DOI was again measured in terms of inward current generated. The EC_{50} was 2.9 ± 0.2 nM, with a maximum effect of 100 ± 6 pA occurring at 100 nM (n = 5; Fig. 2*A*). The depolarizing action of both 5-HT and DOI was antagonized by the 5-HT$_2$ antagonist ketanserin (1 μM; Leysen et al., 1981).

Under voltage clamp, DOI (100 nM) caused an inward current at −60 mV; The current amplitude decreased at more negative

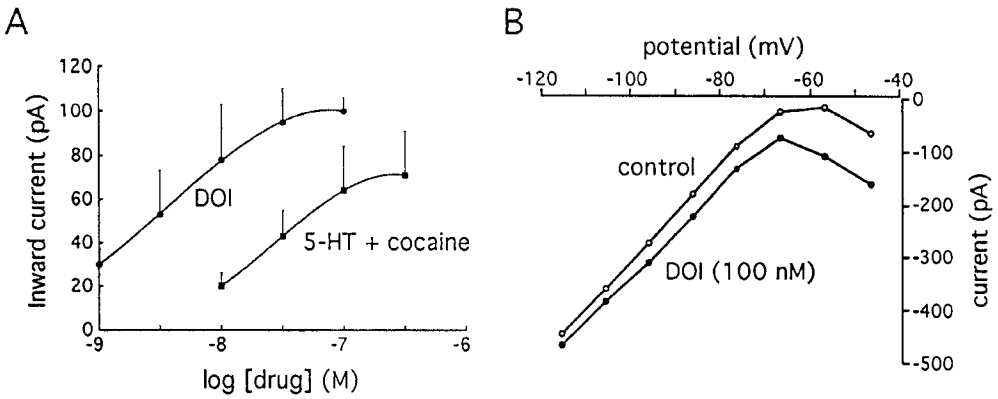

Figure 2. 5-HT and DOI caused inward currents. *A*, Concentration–response curves were generated by determining the amount of hyperpolarizing current needed to offset the drug-induced depolarization (inward current). Membrane potential was −65 to −70 mV. 5-HT (*squares*) was applied in the presence of PBD (1 μM) to block the hyperpolarization and cocaine (10 μM) to block reuptake. DOI (*circles*) was applied with no added drugs. The EC_{50} values for 5-HT and DOI were 21 and 2.6 nM, respectively. Data points represent from three to five determinations. Error bars represent SEM. *B*, Current–voltage relationship for DOI (*solid circles*), as compared with control (*open circles*) was determined under voltage clamp. DOI caused a nonreversing inward current and a slight increase in cell resistance. Holding potential was −57 mV, with hyperpolarizing and depolarizing step increments of 10 mV and 500 msec. Current was measured at the end of the step.

potentials, but did not reverse polarity (steps to −140 mV). The current–voltage relationship demonstrated a slight decrease in cell slope conductance in DOI, as compared with control (Fig 2*B*).

Evoked synaptic potentials

Focal electrical stimulation generated a series of postsynaptic potentials (PSPs). Fast PSPs (duration of 100–200 msec) were caused by the release of glutamate and GABA (D. H. Bobker, unpublished observations). These did not significantly affect the slower synaptic potentials and were therefore not blocked. The fast PSPs were succeeded by a large-amplitude (10–30 mV) IPSP with a duration of 0.5–1.5 sec and due to 5-HT activation of 5-HT$_{1A}$ receptors (Bobker and Williams, 1990b). In the majority of cells (30 of 32), this was followed by a small-amplitude (2–10 mV) s-EPSP with a duration of 30–50 sec.

The s-EPSP amplitude was graded with stimulus intensity (single stimuli delivered at 0.5–5 mA). This was true for the IPSP as well and the two synaptic potentials would vary concurrently with the stimulus strength. At the maximal stimulus, the IPSP was consistently larger than the s-EPSP, with an amplitude ratio (IPSP amplitude:s-EPSP amplitude) of 6.7 ± 0.4 (n = 23; range, 3.0–10.0). At low stimulus strength, it was frequently possible to obtain an IPSP without an associated s-EPSP. In contrast, the s-EPSP could not be isolated by changes in either stimulus strength, position of the stimulating electrodes, or number of stimuli delivered (i.e., trains of 3–10 pulses at 10 Hz).

The s-EPSP was blocked by both TTX (1 μM) and $CoCl_2$ (2 mM; n = 3). With TTX washout, the s-EPSP recovered completely; $CoCl_2$ washout led to a partial (approximately 50%) recovery.

Pharmacology of the s-EPSP

Isolation of the s-EPSP. Several 5-HT$_{1A}$ antagonists were tested to determine whether pharmacological separation of the IPSP and s-EPSP could be obtained. Spiperone (1 μM), NAN-190 (0.3 μM), and cyanopindolol (1 μM) all caused a significant inhibition (>70%) of the IPSP associated with a complete blockade of the s-EPSP. PBD (1 μM) caused an 80 ± 7% (range, 60–95%) inhibition of the IPSP, while reducing the s-EPSP by only 23 ± 7% (n = 5; range, 0–39). PBD had no effect on membrane potential and did not significantly change the s-EPSP duration or time constant of decay (Table 1). PBD (1 μM) was therefore used when isolation of the s-EPSP was desired.

Antagonism of the s-EPSP. To determine if the s-EPSP was mediated by 5-HT$_2$ receptors, two antagonists were used. As noted, spiperone (1 μM) caused a complete and irreversible block of the s-EPSP. Ketanserin (0.1–1 μM) also caused a concentration-dependent inhibition of the s-EPSP, but had no effect on membrane potential itself. Complete inhibition occurred at 1 μM after 5–10 min of superfusion and was irreversible with up to 1 hr of drug washout (Fig. 3). Neither the α1 adrenoceptor antagonist prazosin (1 μM) nor the D_2 dopamine receptor antagonist sulpiride (1 μM) had an effect on the s-EPSP (n = 2 for both).

Table 1. Effect of PBD and cocaine on the s-EPSP

Parameter	Control	PBD (1 μM)	% Change (from control)	PBD + cocaine (1 μM for both)	% Change (from PBD)
Amplitude (mV)	3.1 ± 0.6	2.4 ± 0.4	−23	5.0 ± 1.4	108
TTP (sec)	4.0 ± 0.4	4.3 ± 0.3	8	6.6 ± 0.5	53
Duration (sec)	48 ± 5	45 ± 3	NS	52 ± 4	NS
τ_1 (sec)	ND	1.6 ± 0.4	ND	2.0 ± 0.3	NS
τ_2 (sec)	22 ± 2	18 ± 2	NS	17 ± 2	NS

All data were obtained from five paired cells. ND, not determined; NS, difference not significant.

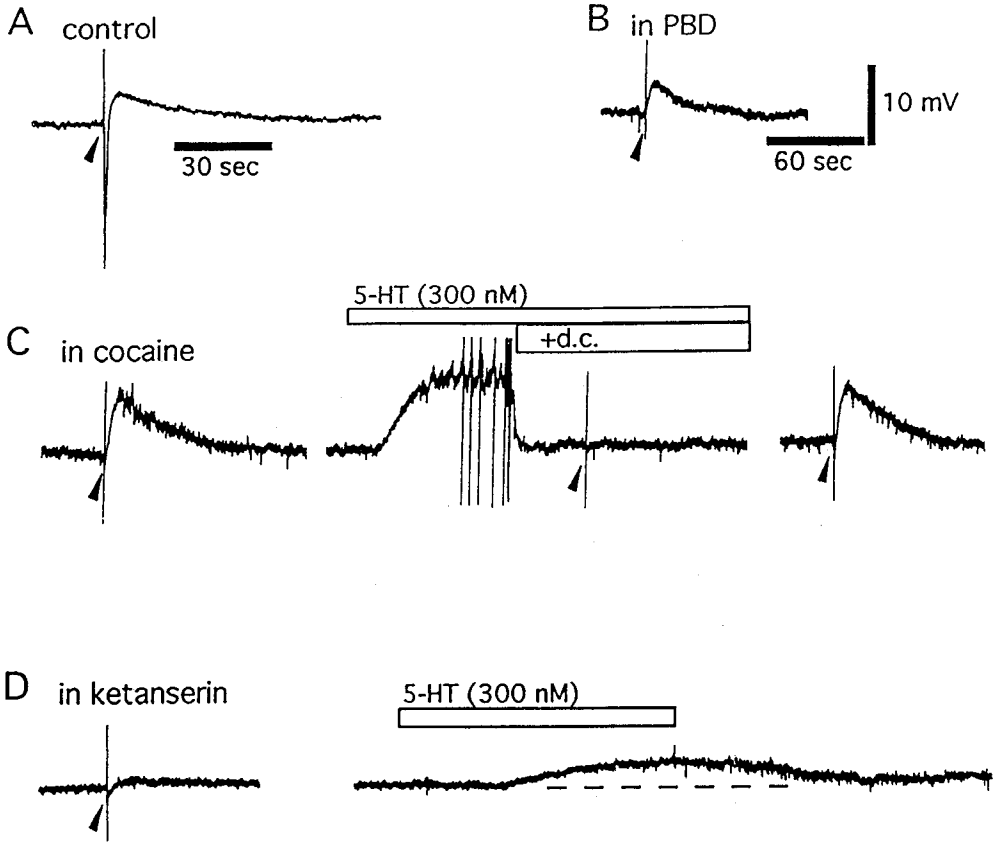

Figure 3. The s-EPSP is due to the action of 5-HT on 5-HT$_2$ receptors. *A*, Voltage recording demonstrates the synaptic potentials caused by a focal electrical stimulus (at *arrowhead* in all traces; initial upward deflection is stimulus artifact). After the artifact, the IPSP lasted about 1 sec, followed by an s-EPSP. Membrane potential was −70 mV. *B*, After PBD (1 μM) was perfused (present for all remaining traces), the s-EPSP could be studied in isolation. Note that the recording speed for traces *B–D* is slower, with the scale at *upper right*. *C:Left*, Cocaine (1 μM; present for all remaining traces) increased the s-EPSP amplitude. *Middle*, 5-HT (300 nM) was applied, causing a depolarization, and the cell began to fire action potentials (fast upward deflections, truncated); hyperpolarizing current brought the potential back to baseline (*open bar* labeled *+d.c.*) and the s-EPSP could not be evoked (at *arrowhead*). *Right*, After washout of 5-HT (not shown) the potential returned to −70 mV and the s-EPSP could again be evoked. *D:Left*, Ketanserin (1 μM) blocked the s-EPSP. *Right*, Ketanserin markedly attenuated the 5-HT-induced depolarization. All results from a single cell.

Occlusion of the s-EPSP. To support the hypothesis that the s-EPSP was due to evoked release of 5-HT acting upon 5-HT$_2$ receptors, an electrical stimulus was applied to evoke an s-EPSP during the 5-HT or DOI-induced depolarization. During the maximum depolarization caused by 5-HT (300 nM, in PBD and cocaine), no s-EPSP could be evoked; With washout and return to baseline potential, the s-EPSP returned (Fig. 3). When DOI (100 nM, without PBD) was perfused, the s-EPSP amplitude was reduced by 89 ± 13%, while the IPSP amplitude was unaffected (n = 3).

Properties of the s-EPSP

Time course. The s-EPSP amplitude in control was 3.6 ± 0.4 mV (n = 23; range, 1.9–10.1 mV; evoked from a potential of −60 to −70 mV). This compares with the IPSP amplitude of 21 ± 1 mV (n = 23; range, 14–30 mV). In 5 of these 23 cells, PBD was applied. The characteristics of the isolated s-EPSP are seen in Table 1. The most notable effect of PBD was a small reduction in s-EPSP amplitude, with no significant change in time course. The s-EPSP could be fit to a curve using two single-exponential functions describing activation (time constant τ_1) and decay (time constant τ_2). This yielded a τ_1 of 1.6 ± 0.4 sec and a τ_2 of 18 ± 2 sec.

Cocaine, an inhibitor of 5-HT reuptake, was tested for its effect on both the IPSP and the s-EPSP. At 1 μM, the s-EPSP amplitude was increased from 3.92 ± 1.6 to 5.78 ± 1.8 mV (49% increase) and duration increased from 44 ± 2 to 66 ± 2 sec (50% increase). The IPSP amplitude (measured in the same cells) did not change significantly, while duration increased from 1.7 ± 0.3 to 4.9 ± 1.2 sec (188% increase, n = 5). Cocaine was also applied to the five cells above, where the s-EPSP was isolated with PBD (Table 1). These results demonstrate a similar increase in amplitude with no effect on duration. Neither τ_1 nor τ_2 of the s-EPSP was significantly changed by cocaine (Fig. 4).

Voltage dependence. The s-EPSP amplitude was voltage dependent, with the maximum occurring near threshold potential (about −55 mV) and progressively decreasing at more negative

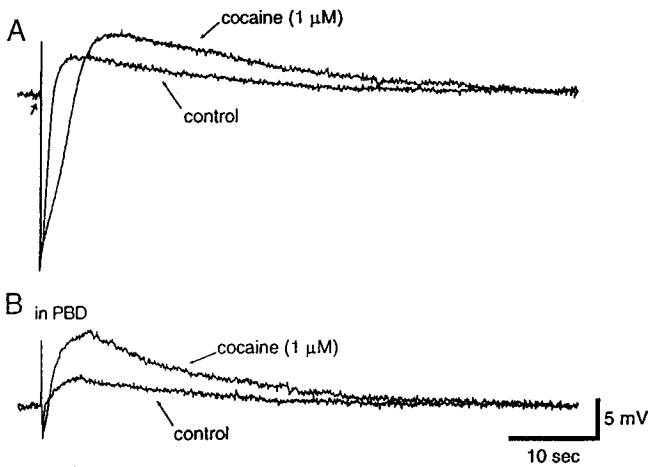

Figure 4. Cocaine affected both the IPSP and s-EPSP. *A*, An IPSP/s-EPSP sequence was evoked by a stimulus (*arrow*). Addition of cocaine (1 μM, *overlapped trace*) increased the duration of the IPSP and the amplitude of the s-EPSP, but had little effect on the s-EPSP duration. Membrane potential was −70 mV. Traces represent the average of three consecutive synaptic potentials. *B*, With the IPSP significantly attenuated by PBD (1 μM), cocaine caused a more pronounced increase in s-EPSP amplitude.

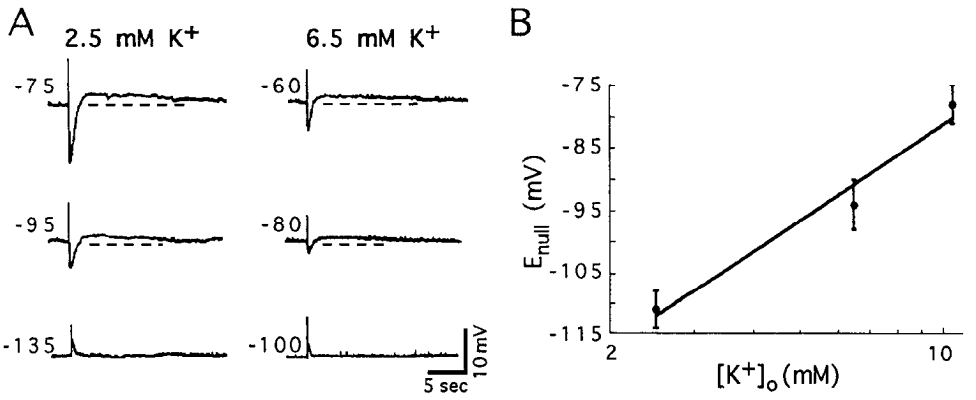

Figure 5. The s-EPSP was voltage dependent. *A*, In 2.5 mM $[K^+]_o$ (*left column of three traces*) synaptic potentials were evoked at the indicated membrane potential (*dashed lines*). The IPSP reversed polarity near the K^+ reversal potential. The s-EPSP did not reverse but became null near the K^+ reversal potential. In 6.5 mM $[K^+]_o$ (*right column*), both the IPSP reversal and the s-EPSP null point shifted to less negative potentials. All traces from as single cell. *B*, Semilogarithmic plot of E_{null} versus $[K^+]_o$ yields a straight line with a slope of 51. Data points are means + SEM for three determinations. $[K^+]_i$ was assumed to be 155 mM.

potentials. The potential at which the amplitude went to 0 (E_{null}) was extrapolated to be -111 ± 3 mV. A reversal of the s-EPSP was not observed at potentials up to -140 mV. By comparison, the IPSP did reverse polarity at a potential of -110 ± 4 mV. Increasing the extracellular potassium concentration to 6.5 mM or 10.5 mM shifted E_{null} of the s-EPSP to -94 ± 4 and -78 ± 3 mV, respectively ($n = 3$). A plot of E_{null} versus $[K^+]_o$ yielded a line with a slope of 51 mV/10-fold change in $[K^+]_o$ (Fig. 5).

Presynaptic inhibition

Previously, we have reported inhibition of the 5-HT–mediated IPSP by activation of presynaptic 5-HT$_{1D}$ receptors (Bobker and Williams, 1990b). To determine if the s-EPSP was subject to the same type of regulation, the 5-HT$_{1D}$ agonist sumatriptan was applied (Humphrey et al., 1988). Sumatriptan (10 nM to 1 μM) caused a concentration-dependent inhibition of both synaptic potentials, with a maximum effect of 80–100% inhibition occurring at 1 μM. The EC_{50} values for inhibition of the s-EPSP and IPSP were 69 ± 3 and 67 ± 38 nM, respectively ($n = 4$; Fig. 6). Sumatriptan did not affect membrane potential or the postsynaptic responses to 5-HT.

Discussion

The aim of the present study was to analyze a 5-HT–mediated s-EPSP observed in type I neurons of the PH nucleus. The significance of this finding is that it supports the hypothesis that 5-HT can act as an excitatory neurotransmitter in the CNS. Previous reports of such an action have included a 5-HT–mediated s-EPSP in spinal motoneurons (5-HT$_2$ receptor; Wang and Dunn, 1990) and a fast EPSP in amygdala (5-HT$_3$ receptor; Sugita et al., 1992). The results presented here are also of interest because of the presence of a 5-HT–mediated IPSP. Other investigators have observed excitatory and inhibitory effects of 5-HT on the same neurons, but have not reported corresponding synaptic potentials (Colino and Halliwell, 1987; Davies et al., 1987; Newberry and Gilbert, 1989; Araneda and Andrade, 1991). The occurrence of the two 5-HT–mediated synaptic potentials on PH neurons affords an unusual opportunity to make comparisons between the responses.

Several lines of evidence indicate that the s-EPSP is caused by 5-HT acting on 5-HT$_2$ receptors. First, ketanserin (an antagonist at 5-HT$_{1C}$ and 5-HT$_2$ receptors) and spiperone (antagonist at 5-HT$_{1A}$, 5-HT$_2$, α1 adrenergic, and D_2 dopamine receptors) acted as potent inhibitors of the s-EPSP. These data coupled with the lack of inhibition by prazosin and sulpiride argue strongly in favor of s-EPSP mediation by the 5-HT$_2$ receptor. Second, the 5-HT$_2$ agonists DOI and 5-HT caused membrane depolarization. Their EC_{50} values of 3 and 21 nM are in good agreement with their affinity for the 5-HT$_2$ receptor as determined from radioligand binding assays (Appel et al., 1990). Third, during the depolarization to both 5-HT and DOI, the s-EPSP could not be evoked. This could have been due to either occlusion of the response, or a presynaptic inhibition. While clearly there is a presynaptic inhibition during 5-HT superfusion, as we have previously demonstrated for the IPSP, no such effect would be expected for DOI. In fact, during DOI superfusion the IPSP was not affected. Finally, cocaine increased the amplitude of the s-EPSP, also supporting a 5-HT–mediated synaptic event.

The ionic mechanism of the s-EPSP was most likely a decrease in conductance to potassium ions. This is supported by two lines of evidence. First, membrane resistance increased during the 5-HT–induced depolarization, suggesting the closing of an ion pore. Second, the s-EPSP amplitude was voltage dependent, becoming null near the potassium reversal potential. With increases in the external potassium concentration, the null point became less negative, in close agreement with the Nernst equation. This is most consistent with the hypothesis that 5-HT inhibited a potassium current that is active at resting potential. Further analysis of the mechanism was complicated by the failure to achieve reversals of the DOI-induced inward current. This may have been due to space-clamp problems, additional currents being generated, or possibly strong rectification of the current. Future experiments will be directed toward examining these possibilities.

The time course of the s-EPSP is consistent with a second messenger–mediated event, being too slow for a directly gated ion channel (Sugita et al., 1992). However, the IPSP is also a second messenger–mediated event and its time course is much shorter than the s-EPSP (Pan et al., 1989; Bobker and Williams, 1990b). There are at least three mechanisms that might account for the long duration of the s-EPSP. First, transmitter could be present within the synaptic cleft for the duration of the s-EPSP. This is unlikely, though, as studies of the isolated IPSP (in ketanserin) demonstrate a total duration of about 1 sec (Bobker and Williams, 1990b). Thus, 5-HT can not be present in the cleft for the 30–50 sec time course of the s-EPSP, unless distinct synapses (i.e., with only 5-HT$_{1A}$ or 5-HT$_2$ receptors) with varying rates of transmitter clearance are proposed. Another mechanism would be second messenger events continuing after agonist has unbound from the receptor. A third explanation is that transmitter unbinds from the two different receptors involved at

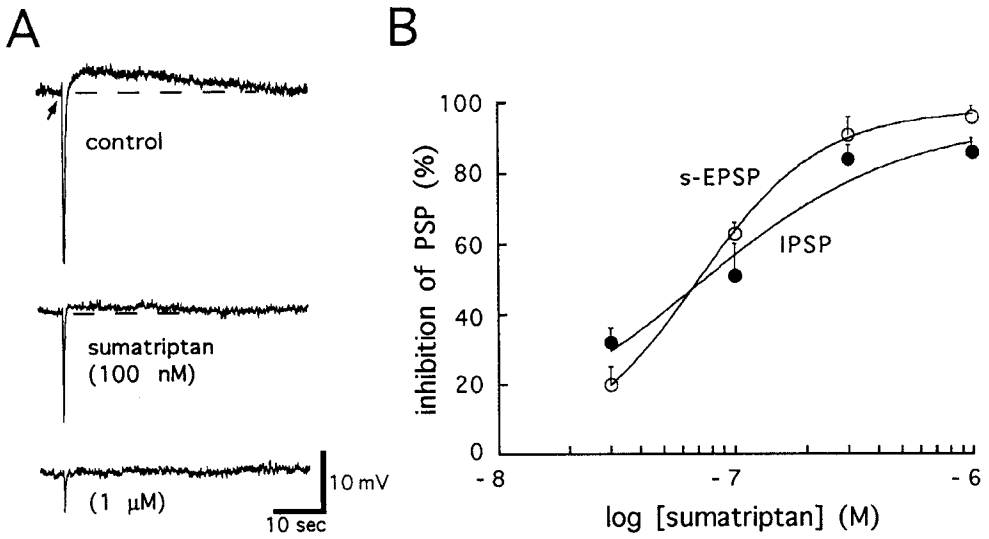

Figure 6. Sumatriptan caused a presynaptic inhibition of both the IPSP and s-EPSP. *A:Upper trace*, Control IPSP/s-EPSP evoked by a stimulus (*arrow*). Membrane potential was −70 mV (*dashed line*). *Lower two traces*, Effect of sumatriptan on the PSPs at 0.1 and 1 μM. Sumatriptan did not antagonize either the hyperpolarizing or depolarizing effect of applied 5-HT. *B*, Concentration–response curve of the inhibition of the s-EPSP (*open circles*) and IPSP (*solid circles*) by sumatriptan.

different rates, as has been shown to account for the time course of glutamate-mediated synaptic potentials (Clements et al., 1992). The experiments conducted here cannot distinguish between the latter two possibilities. It is of interest, however, that cocaine caused a large increase in the duration of the IPSP (presumably by slowing clearance of 5-HT from the synaptic cleft), while causing a relatively small change in the s-EPSP duration. This argues that the s-EPSP duration is determined by factors other than the time transmitter is in the cleft.

Both the s-EPSP and IPSP were inhibited by sumatriptan with nearly identical potencies. This effect occurred at a presynaptic site, as sumatriptan had no direct postsynaptic effects and did not block the postsynaptic actions of 5-HT. The selectivity of sumatriptan for 5-HT_{1D} receptors and a previous study (Bobker and Williams, 1990b) suggest this receptor to be of the 5-HT_{1D} subtype. It is likely, then, that this presynaptic receptor functions as a 5-HT autoreceptor for both the IPSP and s-EPSP.

The physiologic effect of the s-EPSP is to cause a prolonged excitation of PH neurons. The functional importance of this is suggested by previous studies done in the locus coeruleus (LC), which is a major efferent target of the GABAergic neurons in the PH (Aston-Jones et al., 1986; Ennis and Aston-Jones, 1989). It has been reported that systemically administered 5-HT inhibits LC neurons, although direct application into the LC has no effect (Rasmussen and Aghajanian, 1986; Bobker and Williams, 1989b; Done and Sharp, 1992). Gorea and colleagues found that the site of action lay outside the LC, required an intact PH nucleus, and was mediated by 5-HT_2 receptors (Gorea et al., 1991). These reports are consistent with the finding in this study of an excitatory effect of 5-HT on PH neurons, although it has not been demonstrated that the neurons recorded from were GABAergic.

The major finding of this study is that the evoked release of 5-HT can have a direct, long-lasting excitatory effect in the mammalian CNS. This contrasts with the more common finding of an inhibitory (Gerschenfeld and Paupardin-Tritsch, 1974; Pan et al., 1989) or modulatory (acting presynaptically to modify the release of another transmitter; Klein et al., 1982) effect of 5-HT synaptic transmission. Furthermore, it is of interest to note that the evoked release of 5-HT can reproduce the biphasic actions of applied 5-HT, as is seen in other preparations (Davies et al., 1987; Araneda and Andrade, 1991). This supports a role for 5-HT as a neurotransmitter capable of causing opposing postsynaptic effects with varying time courses.

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