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Cyclic AMP and Protein Kinase A Mediate 5-Hydroxytryptamine Type 4 Receptor Regulation of Calcium-Activated Potassium Current in Adult Hippocampal Neurons

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SUMMARY

In the CA1 region of the hippocampus, activation of serotonin receptors of the 5-hydroxytryptamine (5-HT)$_4$ subtype increases membrane excitability by reducing the calcium-activated potassium current responsible for the slow afterhyperpolarization observed in these cells. In the present study, the signaling mechanism by which 5-HT$_4$ receptors reduce the afterhyperpolarization in the CA1 region was examined using intracellular recording in brain slices. Administration of the membrane-permeable cAMP analog 8-bromo-cAMP mimicked the effect of serotonin on the afterhyperpolarization, whereas administration of the protein kinase inhibitor staurosporine inhibited the effects of serotonin. These observations suggested a role for protein kinase A in this response. This was confirmed by intracellular injection of the selective protein kinase A inhibitor Rp-adenosine 3′,5′-cyclic monophosphothioate ((Rp)-cAMPS), which noncompetitively inhibited the ability of serotonin to reduce the after-hyperpolarization. Additional evidence for the involvement of cAMP in the signaling by 5-HT$_4$ receptors was obtained using the general phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine. When this compound was bath administered at concentrations sufficient to enhance a known cAMP-mediated response, a significant enhancement of the ability of 5-HT$_4$ receptors to reduce the afterhyperpolarization was observed. Together, these results indicate that serotonin reduces the afterhyperpolarization in the CA1 region by acting on 5-HT$_4$ receptors that increase intracellular cAMP levels and activate protein kinase A.

Serotonin receptors of the 5-HT$_4$ subtype were originally identified and characterized based upon their ability to stimulate cAMP formation in embryonic collicular neurons in primary culture (1). Subsequent studies have confirmed the ability of these receptors to stimulate adenylyl cyclase in a variety of preparations (2). These results have identified the 5-HT$_4$ receptors as the best understood serotonin receptor subtype capable of coupling to adenylyl cyclase in a stimulatory manner.

Although 5-HT$_4$ receptors are expressed extensively in the brain (3), physiological responses to serotonin mediated through cAMP have been difficult to identify in mammalian neurons. This is in contrast to the well known serotonin modulation of synaptic transmission in *Aplysia*, which involves both cAMP and PKA (4). The best example of a serotonin response mediated by cAMP in mammalian neurons is the serotonin-induced enhancement of a voltage-dependent cation current known as I_h (5, 6). This serotonergic response appears to be mediated by an increase in cAMP, although, surprisingly, the pharmacology of this response suggests that the receptor involved does not belong to the 5-HT$_4$ subtype.

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Previous work has identified a 5-HT$_4$ receptor-mediated response in the CA1 region of rat hippocampus (7–10). In this region, activation of 5-HT$_4$ receptors elicits a slow depolarization and a reduction in the amplitude of the slow calcium-activated AHP that follows the firing of one or more action potentials (7, 10). Because in these cells the AHP normally functions to limit cell responsiveness to strong stimuli, 5-HT$_4$ receptors in this region act to increase cell excitability. Thus, 5-HT$_4$ receptors in this region can be thought of as mediating some of the excitatory responses to serotonin (7, 10).

Although previous pharmacological studies have clearly identified the serotonin receptor responsible for the reduction of the AHP in the CA1 region as belonging to the 5-HT$_4$ subtype (7–9), the initial physiological characterization of this response concluded that this response was not mediated through cAMP (10). This conclusion was based principally on the failure of phosphodiesterase inhibitors to inhibit the 5-HT$_4$ receptor-mediated reduction of the AHP. This result, coupled with additional biochemical and anatomical observations, has led to the suggestion that 5-HT$_4$ receptors might couple to additional second messenger systems in the brain (11). We have therefore reexamined the involvement of the cAMP/PKA cascade in the signaling by 5-HT$_4$ receptors in rat hippocampal slices.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; PKA, protein kinase A; AHP, afterhyperpolarization; IBMX, 3-isobutyl-1-methylxanthine; NE, norepinephrine; (Rp)-cAMPs, Rp-adenosine 3′,5′-cyclic monophosphothioate.

Materials and Methods

Preparation of brain slices. Hippocampal brain slices were prepared using previously published methods (12). Briefly, male albino rats (250–300 g) were anesthetized using halothane and were sacrificed by decapitation. The brains were immersed in ice-cold Ringer solution of standard composition (119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26.2 mM $NaHCO_3$, 11 mM dextrose) and were allowed to cool. The right and left hippocampi were then dissected and brain slices (400-μm nominal thickness) were cut using a vibratome (Lancer Series 1000). The resulting brain slices were then placed on filter paper saturated with Ringer solution, inside a chamber filled with humidified 95% O_2/5% CO_2 at room temperature. After at least 1 hr of recovery, slices were transferred, as needed, to a recording chamber of standard design (13). In this chamber cells were held submerged between two nylon nets and were continuously perfused with Ringer solution bubbled to saturation with 95% O_2/5% CO_2. All experiments were conducted at a temperature of 30 ± 1°.

Electrophysiological recordings. Sharp-microelectrode intracellular recordings were obtained from pyramidal cells of the CA1 region as described previously (8). Cells were impaled with microelectrodes filled with 2 M potassium methyl sulfate (50–120 MΩ), and electrical signals were amplified using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA). All recordings were obtained in the presence of BMY 7378 (3–10 μM), to block the 5-HT_{1A} receptors also present on these cells and thus to pharmacologically isolate the 5-HT_4 receptor-mediated response (7). Tetrodotoxin (500 nM to 1 μM) and tetraethylammonium (5 mM) were also added to the bath. Under these conditions, constant depolarizing current steps (70 msec, 0.6–2.0 nA) trigger all-or-none calcium spikes, followed by a large AHP. Because the most obvious effect signaled by 5-HT_4 receptors in these cells is a reduction of the AHP, these conditions greatly facilitated the analysis of the 5-HT_4 receptor-mediated response.

Most drugs were administered by being dissolved at known concentrations in the bath. In the case of staurosporine, however, pilot studies indicated that its action was extremely slow after bath application (n = 3 cells tested). To circumvent this problem and assess the effect of this compound on serotonin, NE, and 8-bromo-cAMP responses, we compared the effects of these drugs on slices incubated in the presence of staurosporine and under control conditions (mean incubation time, 3.6 hr; range, 1.5–6 hr). (Rp)-cAMPS was dissolved in the electrode solution at a concentration of 1 mM and was injected into the cell by passive diffusion. Because of uncertainties regarding diffusion from the electrode into the cell and diffusion out, it is difficult to estimate the final intracellular (Rp)-cAMPS concentration, although it is probably considerably lower than 1 mM. Pilot studies indicated no effect on NE responses when (Rp)-cAMPS was dissolved in the electrode solution at a 10-fold lower concentration (100 μM, n = 4 cells tested).

Data analysis. Most experiments reported upon in this communication examined drug effects on the amplitude of the AHP. We quantified these effects by determining the peak reduction in AHP amplitude observed after administration of an agonist. This inhibition was expressed as percentage of control, which is a measure of how much the AHP was reduced by the agonist, compared with the control (no-agonist) condition. This procedure was selected because each cell serves as its own control, thus avoiding a large measure of cell to cell variability. Values are reported as mean ± standard error.

To test the effects of staurosporine, it was necessary to compare groups of cells undergoing different treatments. This was accomplished by using a one-way analysis of variance for each agonist, followed by Tukey's *post hoc* protected t test. In the case of the (Rp)-cAMPS experiments, concentration-response curves were established for serotonin and NE using approximately half-log unit concentration steps. Generally, three to five concentrations of the agonist were tested on each cell, with the highest concentration being tested last. This procedure was feasible because no detectable desensitization was evident with the short agonist applications used in this study. The data obtained in these experiments were fitted with a logistic equation using the Levenberg-Marquadt least-squares algorithm, as implemented in Origin (Microcal Software, Northampton, MA). The curve fit was used to obtain values for the maximal effect of the agonist (E_{max}) and the EC_{50}, and t tests were used to compare these values among different groups. To compare the concentration-response relationships for the agonists in the presence and absence of (Rp)-cAMPS, confidence intervals (95%) were constructed around the control curve. Experimental values and fitted curves lying outside this confidence interval were considered to be significant at the 0.05 level.

Results

As reported previously (7–10), administration of serotonin to pyramidal cells of the CA1 region of hippocampus resulted in a reduction of the calcium-activated AHP that follows calcium influx into these cells (Fig. $1A_1$). This effect was most clearly observed after blockade of 5-HT_{1A} receptors, which would normally hyperpolarize the cell membrane and confound interpretation of the response. As illustrated in Fig. 1A, the ability of serotonin to reduce the AHP was antagonized by GR113808, as well as several other selective 5-HT_4 receptor antagonists, indicating that this response is mediated by receptors of the 5-HT_4 subtype (7–9).

If 5-HT_4 receptors act through cAMP to reduce the AHP in these cells, it would be expected that manipulations that increase intracellular cAMP levels should mimic this effect of serotonin. As shown earlier by others (14) and illustrated in Fig. $1B_1$, this is indeed the case. Thus, bath administration of forskolin (15) or the general phosphodiesterase inhibitor IBMX (10, 14) reduced the AHP. Moreover, direct elevation of intracellular cAMP levels by administration of the membrane-permeable cAMP analog 8-bromo-cAMP similarly elicited a reversible reduction in the amplitude of the AHP (15) (Fig. $1B_1$). Administration of the 5-HT_4 antagonist GR113808 failed to reduce the effect of 8-bromo-cAMP, consistent with a direct action downstream from the receptor (Fig. $1B_2$).

The observations outlined above are consistent with the idea that cAMP might mediate the reduction in the AHP signaled by 5-HT_4 receptors in CA1 pyramidal neurons. However, because multiple signaling mechanisms appear capable of reducing the AHP in these cells (16), this mimicry is not sufficient to demonstrate a role for cAMP. Many cAMP-mediated responses, however, involve the activation of PKA. Therefore, we examined the effect of the protein kinase inhibitor staurosporine (17) on the 5-HT_4 receptor-mediated reduction of the AHP. As illustrated in Figs. 2A and 3, the ability of serotonin to inhibit the AHP was reduced in a concentration-dependent manner by staurosporine. This ability of staurosporine to inhibit the 5-HT_4 receptor-mediated reduction in the AHP contrasted with its lack of effect on a response that is also known to be mediated through a G protein but that is independent of kinases, namely the γ-aminobutyric acid type B receptor-mediated hyperpolarization also observed in these cells (18) (Fig. 3).

Staurosporine could inhibit the 5-HT_4 receptor-induced reduction of the AHP either by inhibiting PKA, by inhibiting other kinases such as protein kinase C, or though an effect unrelated to these enzymes. Such effects, however, would not necessarily occur over the same concentration range. Therefore, if the inhibition of the 5-HT_4 response is mediated by an action of staurosporine on PKA, it would be expected that the sensitivity of this response should be comparable to that of other responses known to be mediated by PKA in these same cells.

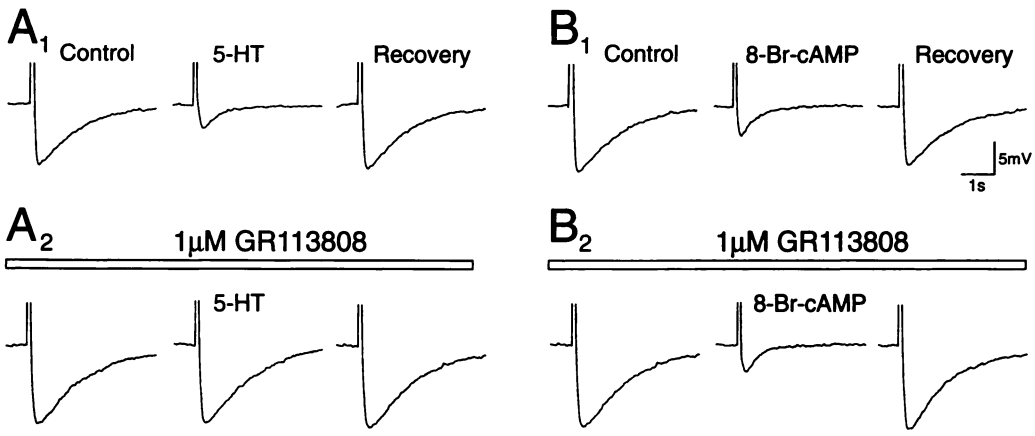

Fig. 1. Reduction of the AHP in CA1 hippocampal pyramidal cells via 5-HT$_4$ receptors. Administration of 10 μM serotonin results in an inhibition of the AHP that follows a calcium spike in these cells (A_1). This effect recovers 15 min after removal of serotonin from the bath. In the same cell, bath application of 1 mM 8-bromo-cAMP mimics the effects of serotonin on the AHP (B_1). Bath application of GR113808 (20 min), a selective 5-HT$_4$ receptor antagonist (3), blocks the effect of serotonin (A_2) but not that of 8-bromo-cAMP (B_2). In this and all subsequent figures, the depolarizing effect of serotonin was neutralized by current injection. As a result, all AHPs were triggered from a common voltage corresponding to the resting membrane potential of the cell. Serotonin was applied in the bath for 2 min and 8-bromo-cAMP for 5 min. The membrane potential for the cell illustrated in this experiment was −64 mV.

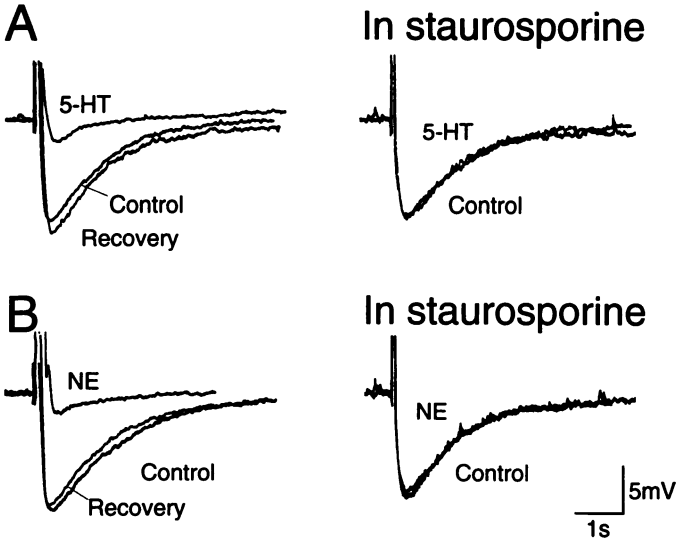

Fig. 2. Inhibition by staurosporine of 5-HT$_4$ and β-adrenergic receptor-induced reductions in the AHP. A, *Left*, superimposed traces illustrating a representative example of the reduction in AHP amplitude elicited by bath administration of 30 μM 5-HT. Cell membrane potential, −65 mV. *Right*, superimposed traces illustrating the effect of 30 μM 5-HT on a slice incubated for 3 hr with staurosporin (10 μM) and maintained in the recording chamber in the presence of 1 μM staurosporin. Cell membrane potential, −61 mV. B, *Left*, superimposed traces illustrating a representative example of the amplitude reduction elicited by 10 μM NE in a control cell. Cell membrane potential, −58 mV. *Right*, effect of 10 μM NE on the AHP in the presence of staurosporine. These records were obtained from the same cell illustrated in *right* for 5-HT. In each experiment, serotonin and NE were applied in the bath for 2–3 min.

The best understood of these is the noradrenergic reduction of the AHP, which in these cells is mediated by β-adrenergic receptors whose activation leads to stimulation of adenylyl cyclase, the formation of cAMP (15), and activation of PKA (19). Therefore, we next compared the potency of staurosporine in inhibiting the effects of serotonin and NE. As illustrated in Fig. 2, when approximately equipotent concentrations of serotonin and NE were tested, staurosporine caused comparable inhibitions in their abilities to reduce the AHP. Moreover, the degree of inhibition obtained using different concentrations of staurosporine was comparable for NE and serotonin (Fig. 3). A similar inhibition by staurosporine was observed when 8-bromo-cAMP was used to bypass the cell surface receptor and directly reduce the AHP (Fig. 3).

To further test the role of cAMP and PKA in signaling 5-HT$_4$ receptor-mediated responses in hippocampus, we tested (Rp)-cAMPS, a selective inhibitor of PKA (20). Because this compound had not been previously injected intracellularly using sharp microelectrodes, we first assessed the effectiveness of the (Rp)-cAMPS injection protocol by examining its effect on β-adrenergic responses. As illustrated in Fig. 4, intracellular injection of (Rp)-cAMPS markedly reduced the ability of NE to reduce the AHP. This inhibition was evident as a significant reduction of the maximal effect of NE (E_{max}), without any change in potency for the agonist. Thus, the maximal possible inhibition of the AHP by NE was reduced from 96 ± 9.3% to 48 ± 3.4% ($p < 0.01$), whereas no apparent change in the NE EC_{50} was detected [control EC_{50}, 1.8 ± 0.6 μM; EC_{50} in the presence of (Rp)-cAMPS, 2.5 ± 0.6 μM].

Having established the effectiveness of this treatment for inhibiting cAMP- and PKA-mediated responses, we then assessed the effects of (Rp)-cAMPS on the 5-HT$_4$ receptor-mediated reduction of the AHP. Concentration-response curves for serotonin were established under control conditions and after intracellular injection of (Rp)-cAMPS. As shown in Fig. 4, the effect of serotonin on the AHP was significantly reduced by intracellularly applied (Rp)-cAMPS. As previously observed with NE, this reduction reflected a decrease in the serotonin E_{max} without a change in its potency. Thus, the maximal inhibition of the AHP possible with serotonin was reduced from 64 ± 9.0% under control conditions to 23 ± 4.2% in the presence of (Rp)-cAMPS ($p < 0.01$), whereas no change was observed in the potency of serotonin [control EC_{50}, 3.3 ± 0.9 μM; EC_{50} in the presence of (Rp)-cAMPS, 3.9 ± 1.9 μM].

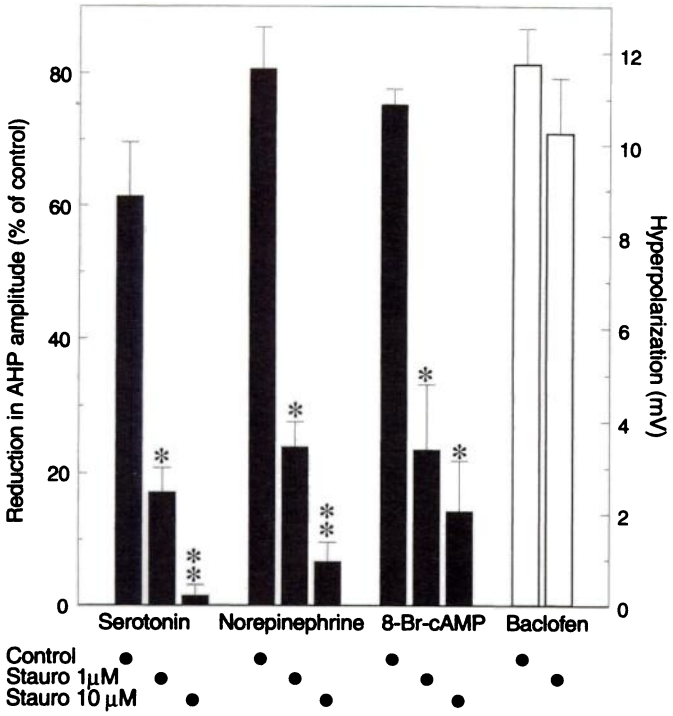

Fig. 3. Comparison of the effects of staurosporine (*Stauro*) on the inhibition of the AHP elicited by serotonin (30 μM), NE (10 μM), and 8-bromo-cAMP (1 mM), as well as the hyperpolarization elicited by baclofen (10 μM). *Error bars*, standard error associated with each group. Serotonin and NE were applied in the bath for 2–3 min. Control serotonin responses were assessed in four cells, serotonin responses in the presence of 1 μM staurosporine in five cells, and serotonin responses in the presence of 10 μM staurosporine in four cells. Control NE responses were assessed in six cells, NE responses in the presence of 1 μM staurosporine in five cells, and NE responses in the presence of 10 μM staurosporine in six cells. Control 8-bromo-cAMP responses were assessed in three cells, 8-bromo-cAMP responses in the presence of 1 μM staurosporine in three cells, and 8-bromo-cAMP responses in the presence of 10 μM staurosporine in four cells. Control baclofen responses were assessed in four cells and baclofen responses in the presence of 1 μM staurosporine in four cells. *, $p < 0.01$ versus control; **, $p < 0.05$ versus 1 μM staurosporine.

The initial characterization of the 5-HT$_4$ receptor-mediated reduction in the AHP in the CA1 region suggested that this response was not mediated by cAMP (10). This conclusion was based upon the failure of the phosphodiesterase inhibitor IBMX to potentiate the 5-HT$_4$ response. In that early study, however, no independent control for the effectiveness of IBMX was conducted. Thus, it is possible that these negative results could have simply reflected insufficient inhibition of the phosphodiesterase in the early experiments. Therefore, we reexamined the effects of IBMX on the 5-HT$_4$ receptor-mediated reduction in the AHP while simultaneously assessing inhibition of the phosphodiesterase by monitoring the enhancement of β-adrenergic responses. This was accomplished by first assessing the effects of low concentrations of serotonin and NE followed by administration of IBMX (100–400 μM). When a concentration of IBMX was found that clearly enhanced the effects of NE, serotonin was reapplied to determine whether its effect was also enhanced. Thus, in each of the cells examined, we assessed the effects of IBMX on both β-adrenergic and 5-HT$_4$ receptor-mediated reduction in the AHP. As illustrated in Fig. 5, in seven cells tested using this procedure administration of IBMX sufficient to clearly enhance β-adrenergic responses (mean enhancement, 22 ± 6.8%; $p < 0.001$) also significantly enhanced the 5-HT$_4$ responses (mean enhancement, 11 ± 2.9%; $p \leq 0.01$). Thus, IBMX, when applied at concentrations sufficient to inhibit the phosphodiesterase, does enhance the ability of 5-HT$_4$ receptors to elicit a reduction in the AHP.

Surprisingly, however, in each of the seven cells tested the enhancement by IBMX of the NE response was considerably larger than the enhancement of the serotonergic response (Fig. 5). Indeed, in this group of cells the enhancement of the NE response by IBMX averaged twice that of the 5-HT response (22% versus 11%), indicating that IBMX was significantly more effective in enhancing β-adrenergic receptor-mediated responses, compared with 5-HT$_4$ receptor-mediated responses ($p < 0.05$). This surprising result suggested that, although both of these receptor types signaled a reduction of the AHP through cAMP, the specific intracellular mechanisms leading to an increase in cAMP were probably not identical.

One possible explanation for the differential effect of IBMX could be that 5-HT$_4$ receptors increase intracellular cAMP by inhibiting phosphodiesterases; this mechanism would render the 5-HT$_4$ receptor-mediated response considerably less sensitive to phosphodiesterase inhibitors. If this were the case, it could be expected that coactivation of 5-HT$_4$ and β-adrenergic receptors would result in a synergistic inhibition of the AHP. To test this possibility, however, it was necessary to first determine the relationship between intracellular cAMP levels and inhibition of the AHP, because nonlinearities in this relationship would affect the interpretation of any test for synergism. This was accomplished by using bath applications of the membrane-permeable cAMP analog 8-bromo-cAMP. As illustrated in Fig. 6B, *inset*, there was a linear relationship between exogenously applied 8-bromo-cAMP and inhibition of the AHP in the concentration range tested ($r = 0.99$, $p < 0.005$). We then examined the additivity of effects triggered by low concentrations of serotonin (3 μM) and NE (1 μM). In four cells tested in this manner, the algebraic sum of the responses elicited by serotonin and NE administered individually approximated that observed when 5-HT and NE were coadministered (Fig. 6). This lack of synergism did not simply reflect saturation in the ability of cAMP to inhibit the AHP, because the response to coadministration of 5-HT and NE was considerably below the maximal inhibition of the AHP possible with either 8-bromo-cAMP or NE (Fig. 6B). Thus, no clear evidence of synergism between serotonin and NE could be detected.

Discussion

The present study used intracellular recording in brain slices to determine the signaling mechanism by which serotonin receptors of the 5-HT$_4$ subtype inhibit the AHP in pyramidal cells of the CA1 region of hippocampus. It was found that the ability of serotonin to reduce the AHP was inhibited in a concentration-dependent manner by staurosporine, suggesting that this effect was mediated by a protein kinase, most likely PKA. This possibility was supported by the use of the selective PKA inhibitor (Rp)-cAMPS, which also markedly suppressed the effects of serotonin. (Rp)-cAMPS inhibited 5-HT$_4$ receptor-mediated responses in a noncompetitive manner, producing a significant reduction in the maximal response to serotonin with little change in its apparent potency. These observations are consistent with an action of (Rp)-cAMPS downstream from

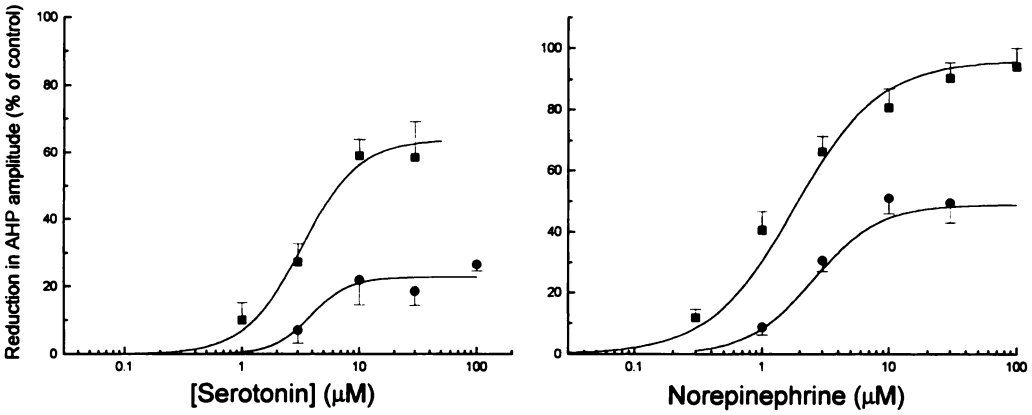

Fig. 4. Effect of (Rp)-cAMPS on the ability of serotonin and NE to reduce the AHP. Intracellular injection of (Rp)-cAMPS inhibited the ability of serotonin and NE to reduce the AHP, without changing the apparent potency of these agonists. *Points*, mean reduction in the amplitude of the AHP obtained at a given concentration; *error bars*, standard error. The *curves* for serotonin and NE in the presence of (Rp)-cAMPS, as well as the mean responses at each concentration tested, were all found to lie outside the 95% confidence interval for the respective control curve and hence were considered to be significantly different at the 0.05 level. ■, Control response (n = 8 cells for 5-HT and 5 cells for NE); ●, response obtained after intracellular injection of (Rp)-cAMPS (n = 7 cells for 5-HT and 4 cells for NE). Serotonin and NE were applied in the bath for 2–3 min for each concentration tested. Applications of this duration were necessary to attain steady state responses (8).

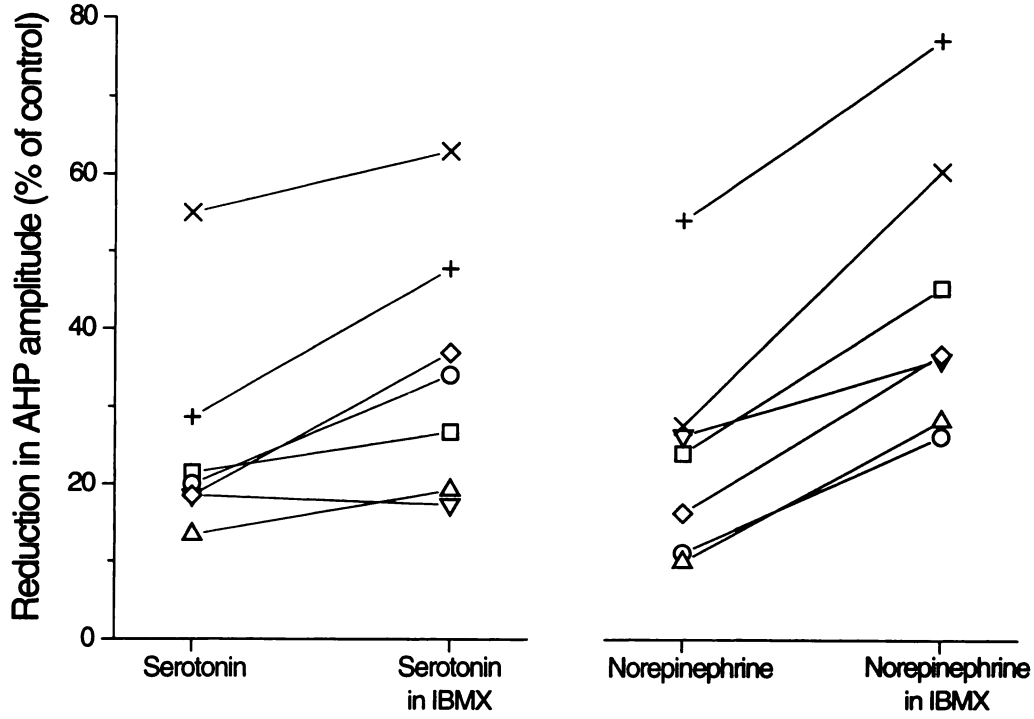

Fig. 5. Comparison of the effects of IBMX on the 5-HT- and NE-induced reductions of the AHP. Seven cells were tested with bath-applied 5-HT (3 μM) and NE (1 μM) before and after application of 100–400 μM IBMX (10–30 min). Serotonin and NE were applied in the bath for 2–3 min each. Each cell is identified by a different *symbol* in the plots.

the receptor, at the level of the signaling mechanism. Consistent with these results, the phosphodiesterase inhibitor IBMX (21), at concentrations that effectively potentiated a known cAMP response, also enhanced the ability of 5-HT$_4$ receptors to reduce the AHP. Thus, these results, together with previous studies showing that a variety of manipulations that increase intracellular cAMP levels also reduce the AHP (14), indicate that 5-HT$_4$ receptors reduce the AHP in CA1 pyramidal cells by increasing intracellular cAMP levels.

Although the 5-HT$_4$ receptor-induced reduction in the AHP was significantly potentiated by IBMX, this effect was small. The small magnitude of the potentiation probably explains the previous failure to detect this effect of IBMX and the conclusion that this response was not mediated through cAMP (10). In any case, given the present results, this early conclusion must now be revised. Although the effect of serotonin was potentiated by IBMX, this potentiation was only about half of that elicited by activation of β-adrenergic receptors, which is known to be mediated through cAMP and PKA (19). The reason for the selective effect of IBMX on the β-adrenergic response is unclear. Because a phosphodiesterase inhibitor would act well downstream from the receptor, at a step common

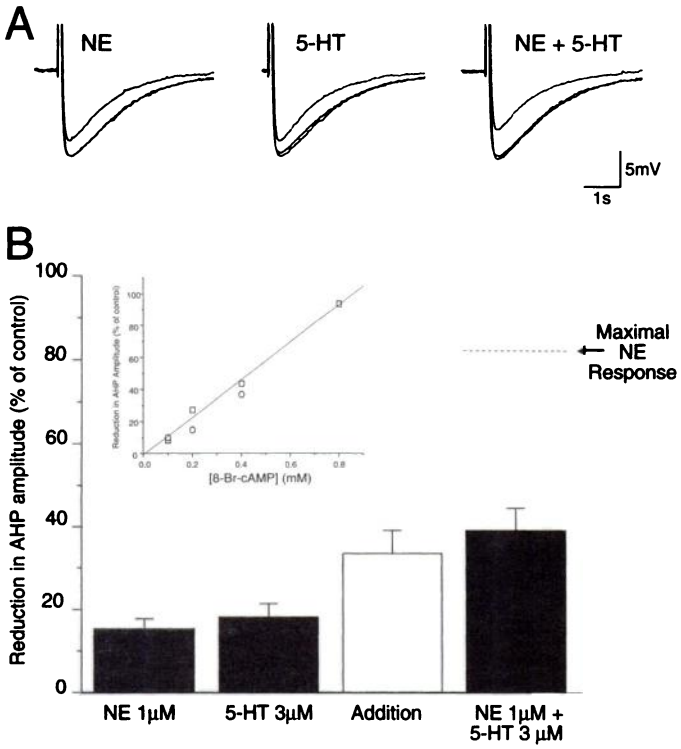

Fig. 6. Test for possible synergism between 5-HT and NE in eliciting a reduction of the AHP. A, Application of NE (1 μM) or 5-HT (3 μM) elicits small reductions in the amplitude of the AHP. Coadministration of NE (1 μM) and 5-HT (3 μM) elicits a larger depolarization that corresponds approximately to the sum of the individual effects of 5-HT and NE. Cell membrane potential, −70 mV. B, The graph summarizes the results obtained in four cells tested as illustrated in A. *Dotted line*, maximal effect of NE as determined in Fig. 4; *error bars*, standard error. Serotonin and NE were applied in the bath for 2–3 min. *Inset*, plot of the relationship between the concentration of bath-applied 8-bromo-cAMP and the reduction in the amplitude of the AHP determined in two cells.

to the signaling by serotonin and NE, it seems unlikely that this difference could be attributed to differences in receptor coupling efficiency or other differences at the receptor level. One possible explanation for these results, however, could be that 5-HT_4 and β-adrenergic receptors both reduce the AHP by increasing intracellular cAMP levels but do so through different mechanisms, β-adrenergic receptors by stimulating adenylyl cyclase and 5-HT_4 receptors by inhibiting phosphodiesterase. A response mediated though an increase in cAMP resulting from stimulation of adenylyl cyclase can be expected to be markedly potentiated by phosphodiesterase inhibitors, as a result of decreased degradation. This was clearly the case for NE in the present experiments. In contrast, a response mediated through an increase in cAMP secondary to phosphodiesterase inhibition can be expected to be relatively insensitive to phosphodiesterase inhibitors, because of occlusion between the receptor response and the enzyme inhibitor. Thus, such a mechanism could explain, in principle, the relative insensitivity of the 5-HT_4 receptor-mediated response to phosphodiesterase inhibition observed in the present experiments. However, if this were the case, it could be expected that 5-HT and NE should act synergistically to inhibit the AHP. No clear evidence of synergy was detected in the present experiments. Thus, it seems unlikely that 5-HT_4 receptors increase intracellular cAMP levels solely by inhibiting phosphodiesterases.

It is still possible that 5-HT_4 receptors might both stimulate adenylyl cyclase and inhibit one or more phosphodiesterases. This could explain the lesser sensitivity of the 5-HT_4 response to enhancement by phosphodiesterase inhibition. Unfortunately, this possibility is hard to test in a convincing manner using the current electrophysiological protocol, because any synergism would be expected to be very small. In this regard, it is noteworthy that a small supra-additive effect was observed in all four cells tested in the present experiment, although this effect could not be adequately resolved from the intrinsic variability of the experiments. Alternatively, it is possible that both 5-HT_4 and β-adrenergic receptors stimulate adenylyl cyclase and that the preferential effect of IBMX on β-adrenergic responses simply reflects differences in the intracellular organization of key components of the signaling cascade that make cAMP generated by this pathway more susceptible to degradation.

The conclusion that 5-HT_4 receptors in the CA1 region signal a decrease in the amplitude of the AHP though cAMP and PKA is consistent with previous biochemical studies indicating that these receptors increase cAMP in guinea pig hippocampus (1, 22). It is also consistent with electrophysiological studies in atrial myocytes (23) and embryonic collicular neurons in culture (24), where these receptors have been shown to elicit their electrophysiological effects through cAMP and PKA. Interestingly, whereas 5-HT_4 receptors regulate a calcium-activated potassium current in the CA1 region, in heart and embryonic collicular they regulate calcium- and voltage-dependent potassium currents, respectively. Thus, 5-HT_4 receptors acting though cAMP and PKA can regulate a diverse group of ionic currents, depending on the cellular background.

Calcium-activated potassium channels are a ubiquitous target for serotonin regulation in the vertebrate central nervous system (25–29). At least three separate serotonin receptors, belonging to different families, have been reported to mediate this regulation (8, 12, 29). In rat prefrontal cortex, the calcium-activated potassium current responsible for the AHP seen in these cells is inhibited by receptors of the 5-HT_2 subtype, presumably acting through protein kinase C (29). This contrasts with the situation in the CA1 region of adult hippocampus, where the calcium-activated potassium current is inhibited by receptors of the 5-HT_4 subtype acting through cAMP and PKA, as well as receptors of the 5-HT_{1A} subtype acting through an as yet unidentified mechanism (12). Thus, there is considerable convergence of different serotonin receptor pathways upon this membrane mechanism. Interestingly, it has recently been reported that, in early postnatal rats, serotonin is also capable of reducing the AHP in CA1 pyramidal cells (19). This response, however, requires much lower concentrations of serotonin and, unlike the 5-HT_4 response seen in adult neurons, can produce a complete suppresion of the AHP. Because this response is inhibited by (Rp)-cAMPS and thus is probably mediated by cAMP, it is possible that the response might be mediated by a receptor of the 5-HT_4 subtype. If that is the case, these results would suggest that 5-HT_4 responses in this area are dramatically regulated during development. Alternatively, it is possible that the response might be mediated by a different serotonin receptor coupled to adenylyl cyclase, possibly of the 5-HT_{1A}, 5-HT_6, or 5-HT_7 subtype. This would require a developmental switch in the expression of serotonin receptors in this area. Further studies will be required to distinguish between these possibilities.

In conclusion, serotonin receptors of the 5-HT$_4$ subtype signal a reduction of the AHP in pyramidal cells of the CA1 region by stimulating intracellular cAMP accumulation and activating PKA. The pharmacological identification of 5-HT$_4$ receptors, as well as the elucidation of their signaling mechanisms in an easily studied, central neuronal preparation, should allow rapid progress in our understanding of the regulation of these receptors in the adult brain and during development.

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