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Pertussis Toxin-Sensitive Guanine Nucleotide-Binding Protein(s) Couple Adenosine A_1 and 5-Hydroxytryptamine$_{1A}$ Receptors to the Same Effector Systems in Rat Hippocampus: Biochemical and Electrophysiological Studies

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SUMMARY

Distinct membrane receptors that elicit similar cellular responses may share elements of signal transduction. In the present study, rat hippocampal adenosine (AD) and 5-hydroxytryptamine (5-HT) receptors were chosen to test this possibility using biochemical and electrophysiological techniques. Responses elicited by the AD receptor that mediates the inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes and hyperpolarization of resting membrane potential (RMP) in rat hippocampal pyramidal cells were characterized and compared, in the same preparation, with those analogous responses elicited by the 5-HT$_{1A}$ receptor. A series of AD agonists including the selective AD A_1 agonist (*R*)-phenylisopropyladenosine [(R)-PIA] inhibited forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes in a concentration-dependent manner. Cyclopentyltheophylline (CPT), a selective AD A_1 antagonist, was a potent, competitive antagonist of this response with a dissociation constant (K_b) of 6 nM (Schild analysis). The rank order of agonist EC_{50} values and antagonist K_b values, as well as stereoselectivity, are consistent with the classification of this receptor as the AD A_1 receptor. Spiperone, a potent 5-HT$_{1A}$ antagonist, competitively antagonized 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes with a K_b value of 14 nM. Intracellular recording techniques revealed that AD, (*R*)-PIA, 5-HT, and 5-carboxyamidotryptamine (5-CT) elicited concentration-dependent hyperpolarization of RMP within the same hippocampal pyramidal cell. The maximal hyperpolarization obtained for the AD or 5-HT analogs was the same for individual pyramidal cells. CPT and spiperone antagonized the hyperpolarization by (*R*)-PIA and 5-CT, respectively. Saturating concentrations of spiperone failed to antagonize (*R*)-PIA-mediated responses and CPT did not block responses elicited by 5-HT in either the biochemical or electrophysiological preparations. The combination of saturating concentrations of 5-HT and (*R*)-PIA evoked nonadditive biochemical responses relative to those observed with (*R*)-PIA alone. Similarly, electrophysiological experiments conducted under voltage-clamp conditions demonstrated that maximally effective concentrations of AD and 5-CT exhibited nonadditive behavior. Because the amount of outward current elicited when these agonists were coperfused was significantly less than the algebraic sum of the currents evoked individually by these agents, we infer that a population of AD A_1 and 5-HT$_{1A}$ receptors activates a common pool of guanine nucleotide-binding proteins. Central administration of pertussis toxin produced similar, dose-dependent reductions in 5-HT- and (*R*)-PIA-mediated inhibition of forskolin-stimulated adenylyl cyclase activity and hyperpolarization of RMP. In summary, these results demonstrate that AD A_1 and 5-HT$_{1A}$ receptors are colocalized on the same hippocampal pyramidal cells and suggest that a common pool of pertussis toxin-sensitive guanine nucleotide-binding proteins couple these receptors to both the reduction of adenylyl cyclase activity and hyperpolarization of RMP.

Membrane receptors that coexist on a neuron and mediate the same functional response may share signal transduction mechanisms. There are several reports of receptors sharing components of these mechanisms in the rat central nervous system with interactions observed at the following three distinct loci: (i) the receptor recognition site, (ii) the G protein, and (iii) the effector system. Somatostatin and muscarinic receptors have been shown to share a common binding domain in rat hippocampal membranes (1). Andrade *et al.* (2) have

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ABBREVIATIONS: G protein, guanine nucleotide-binding protein; ACSF, artificial cerebrospinal fluid; AD, adenosine; 5-CT, 5-carboxyamidotryptamine; CHA, cyclohexyladenosine; CRC, concentration-response curves; CPT, 8-cyclopentyltheophylline; 5-HT, 5-hydroxytryptamine; GABA, γ-aminobutyric acid; PIA, phenylisopropyladenosine; PT, pertussis toxin, PAA, phenylaminoadenosine; RMP, resting membrane potential; NECA, *n*-ethylcarboxamidoadenosine; EGTA, ethylene glycol bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

demonstrated that a PT-sensitive G protein couples 5-HT_{1A} and $GABA_B$ receptors to the same K^+ channels in rat hippocampal pyramidal cells. In cerebellar granule cell membranes, AD A_1 and $GABA_B$ receptors have been reported to share catalytic subunits of adenylyl cyclase (3).

Adenosine and 5-HT receptors evoke similar cellular responses in rat hippocampal preparations, including the inhibition of adenylyl cyclase activity (4–7) and hyperpolarization of RMP (2, 8–12). AD A_1 receptors have been studied in both intact cells and broken membrane preparations, where AD analogs inhibit basal (4, 13, 14) and forskolin-stimulated adenylyl cyclase activity (15, 16) and decrease levels of forskolin-stimulated cAMP (5, 6). In previous studies, the receptor activated by (*R*)-PIA that mediates the inhibition of forskolin-stimulated adenylyl cyclase activity (15, 16) and cAMP levels (5, 6) in neuronal preparations was not characterized using selective AD antagonists. Moreover, the AD receptor that mediates the hyperpolarization of RMP in hippocampal pyramidal cells has not been classified. Therefore, to test whether these receptors share some elements of receptor-mediated signal transduction, it was necessary to characterize the AD A_1 receptor in both the cell-free and slice preparations using the same AD agonists and antagonists. Both the spiperone-sensitive, 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase (7) and 5-HT_{1A}-mediated hyperpolarization of RMP (11, 12) have been characterized previously.

AD A_1 and 5-HT_{1A} receptors were investigated in the present study to test putative sharing of elements of signal transduction in the same neuron. The combination of biochemical and electrophysiological techniques were employed to identify and quantitate AD A_1 and 5-HT_{1A} receptor-mediated responses in rat hippocampal preparations. The results demonstrate that these receptors are colocalized on hippocampal pyramidal cells and suggest that a common pool of PT-sensitive G protein(s) couple the two receptors to both the inhibition of adenylyl cyclase and the hyperpolarization of RMP.

Materials and Methods

Tissue preparation. Hippocampal membranes from male Sprague-Dawley rats (200–250 g) were prepared for measuring adenylyl cyclase activity as previously reported (7). Briefly, tissue was diluted (1:53, wet w/v) in ice-cold buffer (300 mM sucrose, 1 mM EGTA, 5 mM EDTA, 5 mM dithiothreitol, and 20 mM Tris-HCl, pH 7.4 at 22°; STEED) and homogenized in a Teflon/glass homogenizer (20 hand strokes). The homogenate was further diluted 8-fold in STEED and centrifuged at 39,000 × *g* for 10 min at 0°. The supernatant was discarded and the membrane pellet was resuspended in ice-cold STEED to the original diluted volume. Hippocampal membranes were prepared fresh and kept on ice until use. Membrane protein concentrations were determined according to Lowry *et al.* (17) using bovine serum albumin as the standard.

Adenylyl cyclase assay. Adenylyl cyclase activity was assayed in hippocampal membrane homogenates by a modified protocol of De Vivo and Maayani (7), which measures the enzymatic conversion of [α-^{32}P]ATP to [^{32}P]cAMP. To allow measurement of both AD A_1 and 5-HT_{1A} receptor-mediated responses in the same hippocampal membrane preparation, the reaction buffer was modified as follows: (i) addition of AD deaminase, (ii) omission of theophylline, and (iii) elevation of cAMP from 1 mM to 2 mM. Initial studies demonstrated that the response parameters (EC_{50}, E_{max}, and slope index) obtained with 5-HT or (*R*)-PIA, a selective AD A_1 agonist (13, 18, 19), were not significantly different in either the absence or presence of 100 μM rolipram, a nonxanthine phosphodiesterase inhibitor. Moreover, the response parameters for 5-HT and the K_b of spiperone (see Results) were similar to those reported previously by De Vivo and Maayani (7). Therefore, experiments were conducted in reaction buffer lacking rolipram. Preliminary studies demonstrated that CPT, a selective AD A_1 antagonist (20), in the absence of AD deaminase, increased forskolin (10 μM)-stimulated adenylyl cyclase activity. Additionally, EC_{50} values for (*R*)-PIA were lower when AD deaminase was excluded from the reaction buffer. These results demonstrated that endogenous AD is a "contaminant" in the membrane preparation and necessitated the use of AD deaminase in the adenylyl cyclase assay (14), thus preventing the use of AD, the endogenous ligand for the AD receptor, in the biochemical studies.

The adenylyl cyclase assay was initiated by the addition of a 50-μl aliquot of membrane homogenate (25–50 μg) to 200 μl of preincubated (5 min at 30°) reaction mixture (pH 7.4) containing (mM): forskolin (0.01), Tris (80), NaCl (100), GTP (0.01), cAMP (2), ATP (0.2), sucrose (60), EGTA (0.2), EDTA (1), dithiothreitol (1), magnesium acetate (2), and phosphocreatine (5), 10 μg of creatine phosphokinase, 2 units/ml AD deaminase, 1 μCi of [α-^{32}P]ATP, and varying concentrations of drugs. The reaction was incubated an additional 5 min at 30°, at which point the reaction was terminated by the addition of 100 μl of 1% (w/v) aqueous sodium dodecyl sulfate. Approximately 20,000 cpm of [^{3}H]cAMP was added to monitor recovery of [^{32}P]cAMP, which averaged 75%. Purification of the cAMP was performed by sequential elutions from a Dowex AG 50 W-X4 (200–400 mesh) and an Al_2O_3 column (21). Radioactivity was measured by liquid scintillation counting (Beckman Model LS9000). Raw data (cpm) were directly transferred to an IBM/AT computer, which performed curve-fitting functions using the IBM-compatible RS/1 package as outlined below.

The rate of forskolin (10 μM)-stimulated adenylyl cyclase activity in rat hippocampal membranes was constant from 1 to 15 min and was dependent linearly on protein concentration (5–100 μg) in both the absence and presence of 10 μM (*R*)-PIA. The incubation time (5 min) and protein concentration (20–50 μg/assay) used in this study were within these linear ranges. Similar results were reported in this preparation for 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase activity (7).

Electrophysiology of the hippocampal slice preparation. Male Sprague-Dawley rats were anesthetized with ether (Aldrich, Milwaukee, WI) until unconscious and were decapitated. The brain was removed rapidly and rinsed in ice-cold ACSF containing (mM): NaCl (124), KCl (3), NaH_2PO_4 (1.25), $MgSO_4$ (2), $NaHCO_3$ (24), dextrose (10), and $CaCl_2$ (2.5). The right hippocampus was dissected free and transferred to a MacIlwain tissue chopper. Starting at the dorsal/septal tip, 400–500-μm sections were cut and placed in a holding vial containing ACSF at room temperature (25°) that was constantly bubbled with 95% O_2/5% CO_2 to maintain a pH of 7.4. After letting the slices sit for at least 1 hr, one slice was transferred to a recording chamber where it was totally submerged and constantly superfused with aerated (95% O_2/5% CO_2) and heated (34 ± 1°) ACSF at a flow rate of 1–1.5 ml/min.

Intracellular recordings from CA1 pyramidal cells were obtained using standard intracellular recording techniques. Electrodes were pulled from Omega-dot glass capillary tubing (0.6-mm i.d./1.2-mm o.d.; Sutter Instruments) on a Brown and Flaming electrode puller. Electrodes were filled with 2 M KCl and had a resistance of 40–100 MΩ. Neurons were impaled by briefly passing a large depolarizing current or by briefly increasing the capacitance compensation through the tip of the electrode. Hyperpolarizing current of approximately 1 nA was passed to help "seal" the cells. Electrical signals were amplified by an AXOCLAMP II amplifier and recorded on both a Gould chart recorder (Series 2200) and a Vetter Instruments modified videocassette recorder.

Drugs were tested by superfusion with ACSF containing a known concentration of ligand. A concentration of ligand, usually 100-fold greater than the final concentration, was injected directly into a holding syringe containing ACSF at a known volume, to obtain the desired concentration. Perfusion with ligand was continued until a steady state response was obtained, after which the perfusion fluid was switched to ligand-free ACSF. Antagonists were perfused for at least 30 min before

agonist administration or were included in the stock ACSF for the duration of the experiment.

Membrane resistance was measured every 5 sec by quantifying the change in voltage in response to a 100-msec hyperpolarizing current pulse of 300 pA. Current-voltage plots were obtained by measuring voltage changes in response to hyperpolarizing and depolarizing current pulses of 100-pA increments, before, during, and after ligand administration.

To test for response additivity, cells were voltage-clamped using single electrode voltage-clamp techniques. Electrodes were pulled to give a resistance of 40–60 MΩ (2 M KCl). In some experiments, tetrodotoxin (1 μM) was included in the ACSF. The output of the headstage was continuously monitored and the switching frequency was set between 2.5 and 4 kHz. In all experiments, the clamped voltage deviated less than 3 mV. Hippocampal slices were perfused with a maximal concentration of either 5-CT or AD. Once steady state was reached, the other agonist was added to determine whether there was any additional current flow.

PT Administration. Animals were rendered unconscious with ether and maintained surgically anesthetized with methoxyflurane (Pitman-Moore, NJ). Guide cannulas (26 gauge) were stereotaxically placed bilaterally into the hippocampus (guide cannula coordinates: $F = -3.8$ mm; $L = \pm 2.5$ mm, $D = -2.5$ mm) or lateral ventricles (guide cannula coordinates: $F = -0.8$ mm, $L = \pm 1.5$ mm, $D = -3.0$ mm) (22). Bilateral infusions (2 μl each side) of PT or isotonic saline were performed using a 10-μl syringe (Hamilton, Las Vegas, NE) attached to a calibrated syringe pump (Sage Instruments, Cambridge, MA) set at an infusion rate of 1 μl/min. Infusions were made through an injection cannula (33 gauge), the tip of which extended 1 mm beyond the guide cannula. Animals were allowed to recover at least 48 hr before the hippocampus was removed for biochemical and/or electrophysiological studies.

Drugs and chemicals. The following drugs were purchased from RBI (Natick, MA): (*R*)-PIA, (*S*)-PIA, CHA, 2-CA, NECA, PAA, CPT, theophylline, caffeine, and spiperone. Forskolin was acquired from Calbiochem (La Jolla, CA). PT was acquired from List Laboratories (Campbell, CA). [α-^{32}P]ATP (specific activity, 30–40 Ci/mmol) and [^{3}H]cAMP (specific activity, 36 Ci/mmol) were purchased from New England Nuclear (Boston, MA). AD, 5-HT, tetrodotoxin, and all biochemicals used in the adenylyl cyclase reaction mixture and ACSF were purchased from Sigma Chemical Co. (St. Louis, MO). 5-CT was

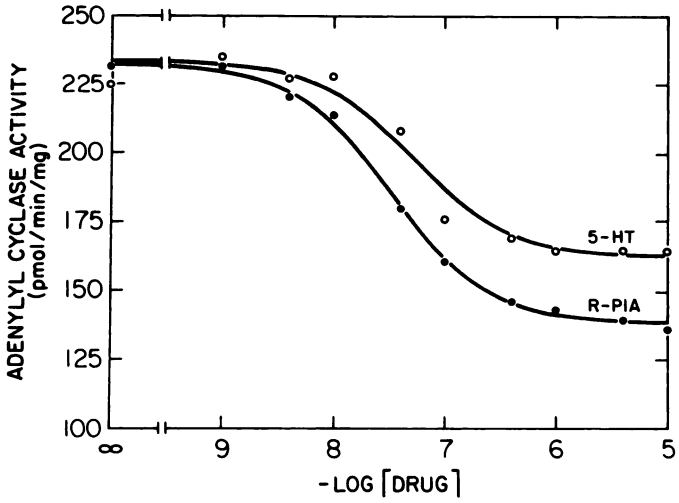

Fig. 1. Representative CRC of the inhibition of forskolin-stimulated adenylyl cyclase activity by (*R*)-PIA and 5-HT in the same rat hippocampal membranes. *Curves* shown were fitted to a two-parameter logistic equation (see Materials and Methods). Calculated EC_{50} and E_{max} values for 5-HT were 52 nM and 31% and for (*R*)-PIA were 33 nM and 41%. The fitted curves each yielded a coefficient of determination (R^2) greater than 0.99. Each *data point* represents the mean of triplicate determinations and standard deviations averaged less than 5% of the mean. The *point* preceding the *break in the curve* represents the mean of three measurements of forskolin-stimulated adenylyl cyclase activity in the absence of agonist (basal activity). Basal forskolin-stimulated adenylyl cyclase activity was 233 ± 8 pmol/min/mg of protein. These results were replicated an additional 10 times, yielding similar results (Table 1).

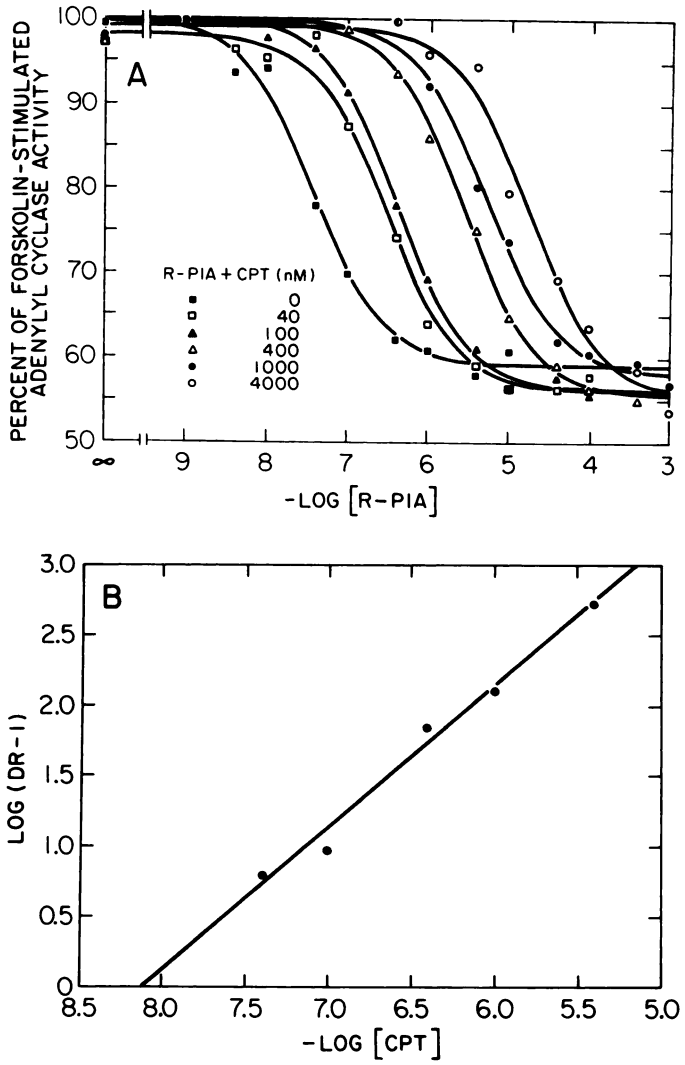

Fig. 2. Competitive antagonism of (*R*)-PIA-mediated inhibition of forskolin-stimulated adenylyl cyclase activity by five concentrations of CPT in rat hippocampal membranes (A). All CRC shown were generated in the same membrane preparation, fit individually to the logistic equation, and normalized to 100% relative to basal forskolin-stimulated adenylyl cyclase activity because of the small decay in stimulated enzyme activity with time. Each *point* preceding the *break in the curve* represents the mean of three measurements of forskolin-stimulated adenylyl cyclase activity in the absence of agonist and antagonist. Basal forskolin-stimulated adenylyl cyclase activity averaged 206 ± 11 pmol/min/mg. CPT, in the absence of (*R*)-PIA, did not significantly affect (<2%) forskolin-stimulated adenylyl cyclase activity relative to basal activity at any of the concentrations tested (40 nM to 4 μM). E_{max} values obtained from the fitted CRC were not significantly different from each other ($p > 0.05$). The coefficients of determination (R^2) of all curves fit to the logistic function were greater than 0.99. Each *data point* represents the mean of triplicate determinations and standard deviations averaged less than 5% of the mean. The K_b value of CPT, calculated by Schild regression (26), was 6 nM (B). *DR* represents the dose ratio and is defined as the EC_{50} value for an agonist in the presence of a known concentration of antagonist divided by the EC_{50} value for the agonist in the absence of antagonist. Linear regression of the data yielded a correlation coefficient (r) of 0.96, a value not significantly different from unity ($p > 0.05$). These results were replicated a total of three times, yielding similar results.

TABLE 1

E_{max} (arithmetic mean ± standard error) and pEC_{50} (mean of −log EC_{50} values ± standard error) values for inhibition of forskolin-stimulated adenylyl cyclase activity by AD agonists in rat hippocampal membranes

CRC data for each drug were fit to the logistic equation to obtain response parameters.

Drug	n^a	E_{max}	pEC_{50}
		% inhibition	
(*R*)-PIA	11	41 ± 1	7.44 ± 0.04
CHA	3	44 ± 2	7.23 ± 0.06
2-Chloroadenosine	3	40 ± 2	6.58 ± 0.03
NECA	5	47 ± 3	6.46 ± 0.05
(*S*)-PIA	3	40 ± 1	6.23 ± 0.10
PAA	3	25 ± 2[b]	4.95 ± 0.11

[a] Number of experimental replications.
[b] Significantly different ($p < 0.01$) from the E_{max} value of (*R*)-PIA determined by Dunnett's *post hoc* test for multiple comparisons.

generously supplied by Dr. W. Feniuk, Glaxo (Ware, England). Rolipram was graciously donated by Dr. S. Topiol of Berlex Laboratories (Cedar Knolls, NJ). All chemicals were of the highest purity available commercially. Forskolin, CPT, and PAA were dissolved in ethanol and diluted such that the assay mixture contained less than 0.02% ethanol. Spiperone was dissolved in 10% acetic acid and neutralized to pH 7.0 before use. All other drugs were dissolved in glass distilled H_2O.

Data analysis. Analyses of CRC were performed by a weighted nonlinear regression analysis program (RS/1; Bolt, Beranck, and Newman, Boston, MA) using an IBM/AT computer. The data were fit to a two-parameter logistic equation to obtain EC_{50} and E_{max} values, as described previously (7):

$$R = R_i\text{-}(R_i\text{-}R_0)/[([A]/EC_{50})+1]$$

where R is the rate of adenylyl cyclase activity (pmol of cAMP/min/mg) in the presence of a specified concentration of agonist, $[A]$; R_0 is the rate of activity in the absence of agonist (i.e., basal forskolin-stimulated activity); R_i is the calculated E_{max} value (rate of maximal inhibition by the agonist); and EC_{50} is the concentration that produces half-maximal inhibition. Insertion of a third parameter (slope factor) as an exponent into the logistic function (23) did not significantly improve the fit of CRC by the partial F test ($p > 0.05$) (24). Consequently, this third parameter was omitted in later calculations (see Results). E_{max} values for adenylyl cyclase activity are expressed as an absolute rate value (pmol/min/mg) or per cent inhibition of forskolin-stimulated adenylyl cyclase activity and represent the arithmetic mean ± standard error. EC_{50} values are expressed as the mean of −log EC_{50} (pEC_{50}) because this response parameter (EC_{50}) is not normally distributed around the mean (25). The K_b value of CPT was determined by Schild regression analysis (26). Apparent K_b values (arithmetic means ± standard error) of other antagonists were determined from a shift of CRC in the presence of one concentration of antagonist. Statistical significance was determined by the Student t test for the paired data and by an analysis of variance (ANOVA) for multiple comparisons followed by Dunnett's *post hoc* analysis (27). Trend line analysis was utilized to determine correlations between parameters. p Values less than 0.05 were considered statistically significant. All experiments were replicated a minimum of three times.

Results

Pharmacological characterization of the AD A_1 receptor in rat hippocampal membranes. CRC for (*R*)-PIA- and 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase activity ("response" to (*R*)-PIA and 5-HT) were generated in the same rat hippocampal membrane preparation (Fig. 1). Three response parameters (EC_{50}, E_{max}, and slope index) were calculated from computer fits of the logistic equation to the concentration response data (Materials and Methods). The E_{max} values of the response to (*R*)-PIA were consistently greater (41 ± 1%) than those of 5-HT (31 ± 1%) using Student's paired t test ($p < 0.01$; 11 experiments). The mean EC_{50} value for (*R*)-PIA (pEC_{50} = 7.44 ± 0.04; EC_{50} = 36 nM) was lower than that obtained with 5-HT (pEC_{50} = 7.09 ± 0.05; EC_{50} = 81 nM). The slope indices of the CRC to (*R*)-PIA (1.06 ± 0.12) and to 5-HT (1.25 ± 0.19) were not significantly different from unity (11 experiments; $p > 0.05$). Therefore, the curves shown in Fig. 1 were computer-fit to the logistic equation with slope index set to unity.

The selective AD A_1 antagonist CPT (20) was used to classify

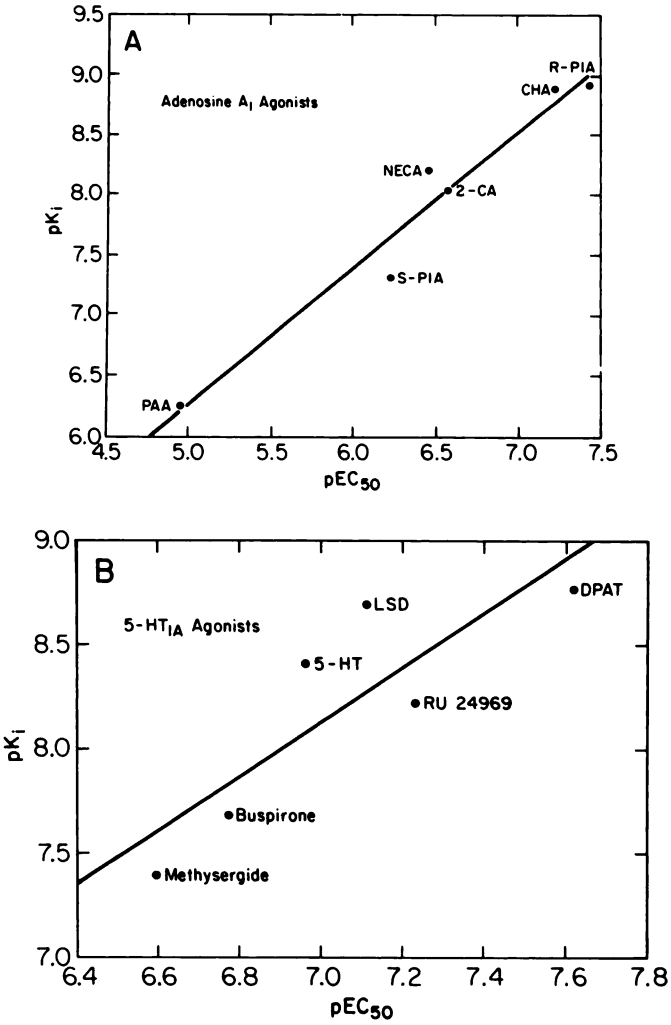

Fig. 3. A, Relation between the pEC_{50} values of AD agonists for the AD receptor mediating the inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes (Table 1) and their pK_i values for the theophylline-sensitive [^{3}H]CHA sites in rat brain membranes (19). The K_i values for the AD agonists were converted to pK_i values. The correlation coefficient (r) of the fit of the line to the data was 0.98 ($p < 0.001$), with a slope of 1.13. B, Relation between the pEC_{50} values of 5-HT agonists for the 5-HT receptor mediating the inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes (7) and their pK_i values for the 5-HT-sensitive [^{3}H]8-hydroxy-2-(di-*n*-propylamine)-tetralin (*DPAT*) sites in rat hippocampal membranes (28). Because the slope indices for the competition curves did not significantly deviate from unity, the IC_{50} values for the 5-HT agonists were converted to K_i values using the Cheng and Prusoff equation (29) and then converted to pK_i values. The correlation coefficient (r) of the fit of the line to the data was 0.85 ($p < 0.05$), with a slope of 1.25. LSD, *d*-lysergic acid diethylamide.

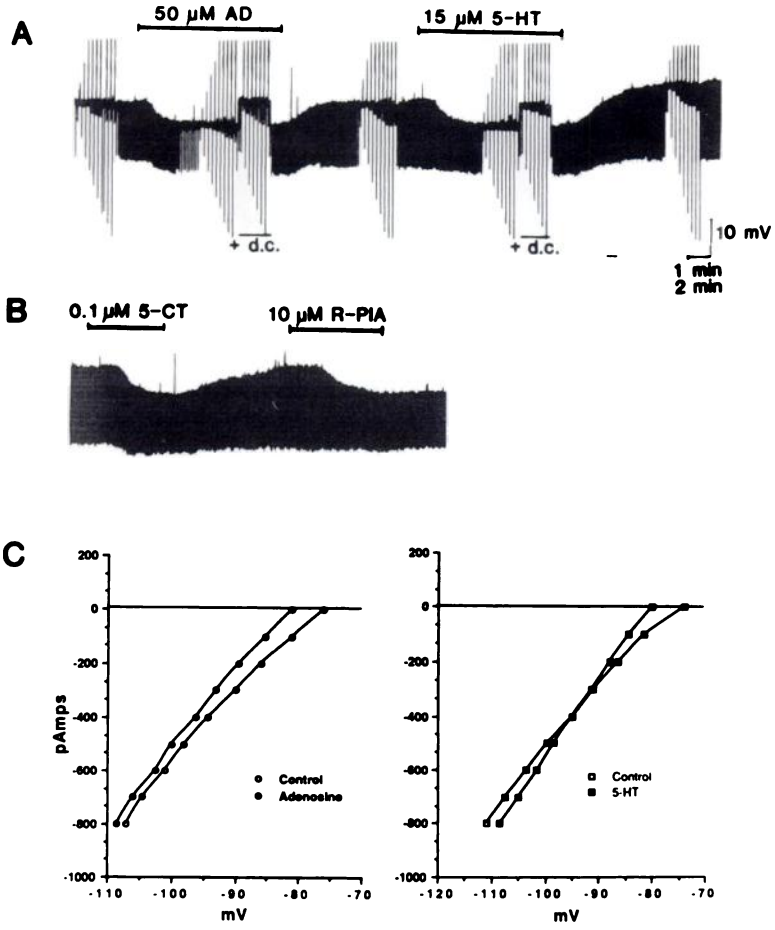

Fig. 4. Chart records of voltage responses to AD and 5-HT in agonists hippocampal pyramidal cells in the CA1 region. The *top tracing* (A) was obtained from a single cell with a RMP of −74 mV. The *middle tracing* (B) was obtained from a different cell with a RMP of −70 mV. Hippocampal slices were perfused with agonists for the length of time denoted by the *solid bar*. Downward and upward deflections represent change in voltage due to intracellular injection of 300-pA, 100-msec hyperpolarizing current pulses or hyperpolarizing and depolarizing current pulses of 100 pA increments. Chart record speed increased during current pulses of varying amplitude (A). During steady state drug effects, the membrane potential was returned to resting level by injection of direct current as indicated (A). C shows the current-voltage plots for the AD and 5-HT responses from A. The voltage obtained in response to hyperpolarizing current pulses of 100-pA increments was measured before drug perfusion and during steady state drug perfusion and plotted as indicated.

TABLE 2

Effects of CPT and PT on responses elicited by perfusion with AD, (*R*)-PIA, 5-HT, and 5-CT in hippocampal pyramidal cells

Intracellular recordings were made from untreated hippocampal slice preparations, hippocampal slices perfused *in vitro* with CPT, or preparations pretreated *in vivo* with PT with the dose as indicated (see Materials and Methods). CPT (1 μM) was perfused for 30 min before and during agonist administration or included in the stock ACSF (see Fig. 5A). Membrane resistance was calculated by measuring the change in voltage in response to a 300-pA 100-msec hyperpolarizing pulse (see Figs. 4 and 5). Values are expressed as arithmetic mean ± standard error. Number in parentheses represent number of cells.

Treatment	RMP	Cell Resistance	Hyperpolarization			
			AD (100 μM)	(*R*)-PIA (10 μM)	5-HT (15 μM)	5-CT (0.1 μM)
	mV	*MΩ*	*mV*			
None	−70.3 ± 1.2 (11)	56.1 ± 2.8 (11)	7.2 ± 0.3 (6)	6.4 ± 0.6 (5)	6.8 ± 1.1 (4)	7.3 ± 0.6 (8)
CPT	−60.4 ± 1.6[a] (7)	88.2 ± 1.6[a] (7)	1.0 ± 1.8[a] (4)	1.0 ± 0.5[a] (5)	ND[b]	10.8 ± 1.0[a] (7)
PT (1 μg)	−66.0 ± 2.5 (2)	54.0 ± 7.0 (2)	ND	10.0 ± 2.0 (2)	8.0 ± 2.0 (2)	ND
PT (4 μg)	−73.0 ± 0.6 (4)	35.0 ± 5.0 (4)	ND	1.7 ± 1.0[a] (4)	1.8 ± 0.8[a] (4)	ND

[a] Values were significantly different ($p < 0.05$) from control (no treatment) values determined by unpaired two-tailed *t* test.
[b] ND, not determined.

the AD receptor mediating the inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes using the agonist (*R*)-PIA. In the presence of five concentrations (40 nM to 4 μM) of CPT, the CRC to (*R*)-PIA were shifted to the right in a parallel fashion with complete surmountability of the antagonism (Fig. 2A). CPT was a potent and competitive antagonist of the (*R*)-PIA-mediated response, with Schild analysis yielding a dissociation constant (K_b) of 6 ± 2 nM (three experiments) with a slope not significantly different from unity ($m = 0.95 \pm 0.05$; $p > 0.05$; Fig. 2B). This dissociation constant value is similar to the K_i value (11 nM) reported for the cyclopentyladenosine-sensitive [^{3}H]8-cyclopentyl-1,3-dipropylxanthine binding sites in rat brain membranes (20). Theophylline (50 μM; apparent $K_b = 6 \pm 2$ μM; three experiments) and caffeine (150 μM; apparent $K_b = 25 \pm 9$ μM; three experiments) were also surmountable and competitive antagonists of the response to (*R*)-PIA in rat hippocampal membranes (data not shown). The K_b values of these three drugs agree with the K_i values for the theophylline-sensitive [^{3}H]CHA binding sites in rat brain membranes (18).

Spiperone (500 nM) antagonized, in a competitive and surmountable manner, the response to 5-HT in rat hippocampal

membranes. The apparent K_b value of spiperone (14 ± 5 nM; three experiments) is consistent with a value reported previously from our laboratory (7). However, 100 μM spiperone failed to affect the response to 10 μM (*R*)-PIA (E_{max} = 40.3 ± 3.3%; four experiments; compare Fig. 1). Likewise, 100 μM CPT did not alter the response to 10 μM 5-HT (E_{max} = 29.9 ± 2.5%; four experiments, compare Fig. 1).

In addition to (*R*)-PIA, five AD agonists were tested in rat hippocampal membranes. Mean values of pEC_{50} and E_{max} are summarized in Table 1. The EC_{50} values of (*R*)-PIA (36 nM) and CHA (59 nM) were 200–300 times lower than that of PAA (11 μM), a selective AD A_2 agonist (19), which also exhibited partial agonist activity (intrinsic activity, 0.6 relative to (*R*)-PIA). Additionally, the EC_{50} value of (*R*)-PIA was approximately 15 times lower than that of (*S*)-PIA (590 nM); this difference is consistent with the results of binding studies in which the PIA enantiomers exhibit approximately 40-fold stereoselectivity for the recognition (binding) site of the AD A_1 receptor (18, 19). Rank order of the EC_{50} values (Table 1) is similar to the rank order of the potencies of these agonists for the AD A_1 binding site in guinea pig and rat brain membranes (18, 19). A high correlation ($r > 0.98$; $p < 0.001$) was obtained for K_i values for these agonists determined on theophylline-sensitive [^{3}H]CHA sites for the AD A_1 binding site in rat brain membranes (19) and EC_{50} values (Table 1; Fig. 3A).

Pharmacological characterization of the AD A_1 receptor in rat hippocampal slice preparation. In the CA1 field of the hippocampal slice preparation, AD and 5-HT produced a membrane hyperpolarization concomitant with a decrease in membrane resistance on the same pyramidal cell (Fig. 4A). The response to AD and 5-HT was rapid (1–2 min), reversible (5–10 min to restore membrane potential upon removal of the ligands from the ACSF), and concentration dependent; AD was effective at concentrations ranging from 3 to 100 μM, whereas 5-HT was active at concentrations between 0.6 and 30 μM. The maximal hyperpolarization elicited by either AD or 5-HT was similar within pyramidal cells, averaging 7 mV (Table 2). The change in membrane resistance following perfusion with 15 μM 5-HT was greater than that observed with 50 μM AD (20.5 ± 4.1 MΩ versus 8.2 ± 3.1 MΩ, $p < 0.05$ by unpaired *t* test; see Fig. 4A). Current-voltage plots, obtained from measuring the membrane potential in response to hyperpolarizing current pulses of 100-pA increments before and during drug perfusion, were constructed for both AD and 5-HT (see Fig. 4C). The lines intersected at approximately −90 mV for 5-HT but did not cross for AD. In some cells, the lines did cross but always at a potential more negative than that observed for 5-HT.

The hyperpolarization elicited by 5-HT has been shown to be mediated by the 5-HT_{1A} receptor (11, 12). Because 5-HT also produces a depolarizing response (seen in Fig. 4A as rebound depolarization) and blocks the Ca^{+2}-dependent K^+ channel mediating the afterhyperpolarization (11, 12), 5-CT, a selective and potent 5-HT_1 agonist (30), was used to elicit the 5-HT_{1A}-mediated hyperpolarization. The hyperpolarization produced by 5-CT was slower in onset than that of 5-HT or AD, concentration dependent (0.006–0.3 μM), slowly reversible (10–30 min to restore membrane potential upon removal of 5-CT from the ACSF), and antagonized by 1 μM spiperone (70 × K_b at the 5-HT_{1A} receptor). The maximal response elicited by 0.1–0.3 μM 5-CT was equal in magnitude to the hyperpolarization elicited by 15 μM 5-HT and 50–100 μM AD (Fig. 4B; Table 2). 5-CT had no effect on the afterhyperpolarization and did not depolarize the pyramidal cell in the absence or presence of 1 μM spiperone (data not shown).

The selective AD A_1 drugs (*R*)-PIA and CPT were tested to identify the receptor(s) mediating the hyperpolarizing response elicited by AD. (*R*)-PIA was effective at a concentration range of 0.6–10 μM and, at maximal concentrations, elicited a hyperpolarization equal in magnitude to that produced by 5-HT, 5-CT, and AD (Fig. 4B; Table 2). The response evoked by perfusion with (*R*)-PIA was slower in onset as compared with 5-HT and AD, was not rapidly reversible (>1 hr), and was observed in the same pyramidal cells that exhibited a 5-HT_{1A} receptor-mediated hyperpolarization (Fig. 4B). Perfusion with 1 μM CPT (100 × K_b for the AD A_1 receptor; see Fig. 2) significantly blocked the hyperpolarization elicited by 10 μM (*R*)-PIA and 50 μM AD, without antagonizing the hyperpolarization elicited by 0.1 μM 5-CT (Fig. 5A; Table 2). The RMP of the cells recorded in the presence of CPT was, on average, 10 mV more depolarized, and the membrane resistance was significantly greater than control cells (Table 2). The magnitude of the hyperpolarization recorded in response to perfusion of 0.1 μM 5-CT with CPT in the ACSF was greater than the control response. In contrast, 1 μM spiperone had no effect on RMP or resistance (data not shown).

Evidence supportive of PT-sensitive G proteins coupling AD A_1 and 5-HT_{1A} receptors to the same effector systems. Intracellular recordings were obtained in hippocampal slices taken from PT (1 and 4 μg)-treated animals. Approximately 50% of these slices were taken from the hippocampus of the same animal assayed for receptor-linked adenylyl cyclase activity. The results are summarized in Table 2. In two cells from an animal pretreated with 1 μg of PT, the voltage response to 5-HT and (*R*)-PIA perfusion was unaltered. The magnitude of the hyperpolarization elicited by 5-HT and (*R*)-PIA was reduced by greater than 75% in animals pretreated with 4 μg of PT (Fig. 5B; Table 2).

Bilateral administration of 2 and 4 μg of PT produced similar reductions in E_{max} values to (*R*)-PIA- and 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase activity (Fig. 6A) without altering the EC_{50} values and the slope indices (data not shown). Additionally, the ratio of the maximal response to 5-HT divided by the maximal response to (*R*)-PIA remained constant (0.64–0.67) following saline and PT treatments (Fig. 6B).

Possible additivity of the biochemical response to saturating concentrations of (*R*)-PIA (1 μM) and 5-HT (1 μM) was investigated in the rat hippocampal membrane preparation. As shown in Fig. 7, the E_{max} values for the response to (*R*)-PIA and 5-HT when tested individually were 42 ± 4% and 29 ± 2%, respectively (four experiments). Assaying the combination of saturating concentrations (1 μM) of 5-HT and (*R*)-PIA (E_{max} = 48 ± 5%) did not significantly ($p > 0.05$) increase the E_{max} value from that observed with (*R*)-PIA alone (four experiments).

To test for additivity of electrophysiological responses following coactivation of 5-HT_{1A} and AD A_1 receptors in intact cells, hippocampal pyramidal cells were voltage-clamped and the outward current in response to perfusion with maximal concentrations of AD and 5-CT was measured. Additivity was measured using one of two experimental paradigms, (i) addition of 5-CT once the AD response had reached steady state or (ii)

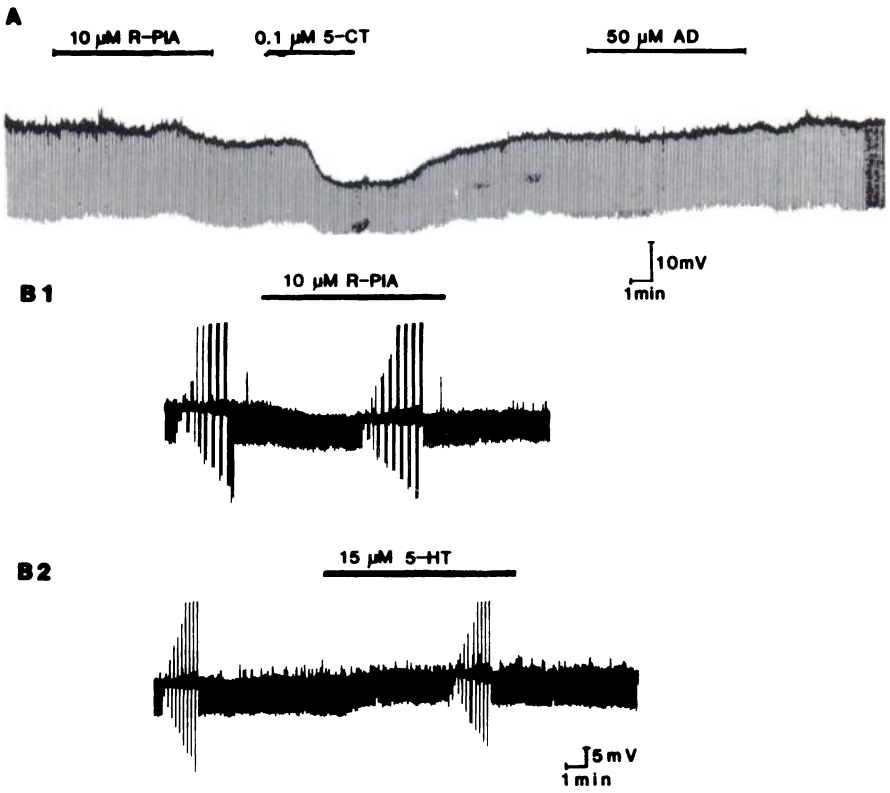

Fig. 5. Effects of CPT and PT on membrane potential of hippocampal pyramidal cells perfused with 5-HT and AD agonists. A, Chart recording of voltage responses of a hippocampal pyramidal cell to perfusion of (*R*)-PIA (10 μM), 5-CT (0.1 μM), and AD (50 μM) in the presence of 1 μM CPT. The record was obtained from a cell with a RMP of −59 mV. CPT was perfused for 30 min before agonist administration and was continuously present in the ACSF during agonist applications. The duration of agonist perfusion is depicted by the *solid bar*. Downward deflections denote change in membrane potential elicited by 300-pA 100-msec hyperpolarizing pulses. B1 and B2, Chart recordings of membrane potential of a hippocampal pyramidal cell taken from an animal treated with 4 μg of PT. The record tracings were obtained from the same cell with a RMP of −75 mV. Hippocampal slices were perfused with agonists for the length of time denoted by the *solid bar*. Downward and upward deflections denote change in membrane potential elicited by 300-pA 100-msec hyperpolarizing pulses or hyperpolarizing and depolarizing current pulses of 100 pA increments to monitor changes in membrane resistance.

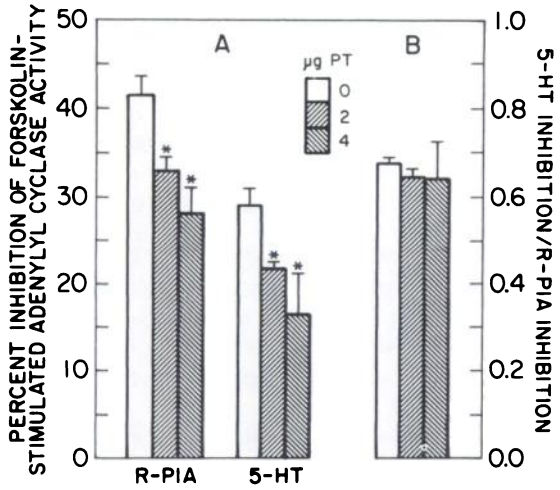

Fig. 6. Effect of PT on (*R*)-PIA- and 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes. CRC (1 nM to 10 μM) for (*R*)-PIA and 5-HT were generated as in Fig. 1 and response parameters (EC_{50}, E_{max}, and slope index) were calculated from data collected from the same hippocampal tissue pretreated *in vivo* with saline or 2 or 4 μg of PT (see Materials and Methods). A, E_{max} values (per cent inhibition) were calculated from the CRC fitted to the logistic equation and were normalized to 100% relative to basal forskolin-stimulated adenylyl cyclase activity (see Fig. 2). B, Ratio values are defined as the maximal response to 5-HT divided by the maximal response to (*R*)-PIA for each experimental condition. Values are expressed as arithmetic mean ± standard error (four experiments). *Asterisks* denote statistical significance ($p < 0.01$) relative to appropriate control group using Dunnett's *post hoc* test for multiple comparisons.

addition of AD once the maximal response to 5-CT had been attained (Fig. 8). The amount of current evoked by 0.3 μM 5-CT was significantly ($p < 0.05$) greater than that observed with 100 μM AD when the agonists were perfused separately (Table

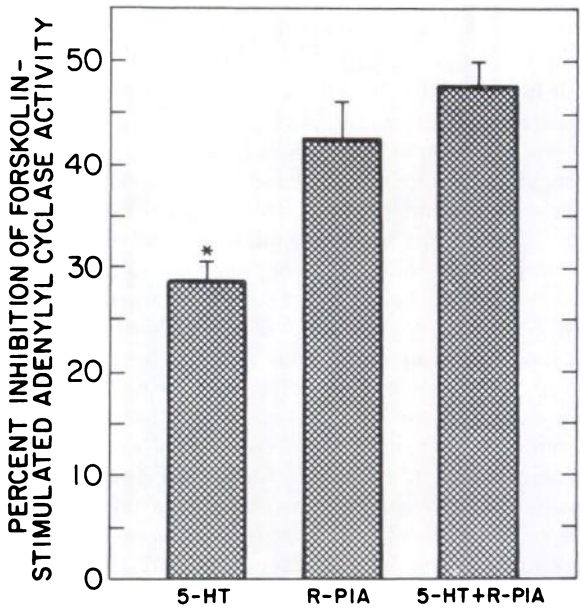

Fig. 7. Nonadditive inhibition of forskolin-stimulated adenylyl cyclase activity by 5-HT and (*R*)-PIA in the same rat hippocampal membranes. Membranes were incubated in the absence and presence of saturating concentrations (1 μM) of 5-HT (15 × EC_{50} value for the 5-HT_{1A} receptor) and/or (*R*)-PIA (25 × EC_{50} value for the AD A_1 receptor). Response depicted is inhibition of forskolin-stimulated adenylyl cyclase activity evoked by the agonist(s). Values were calculated as ratio of adenylyl cyclase activity in the presence of agonist divided by adenylyl cyclase activity in the absence of agonist(s) (basal). Values represent arithmetic mean ± standard error (four experiments). *Asterisk* denotes statistical significance ($p < 0.01$) relative to (*R*)-PIA using Dunnett's *post hoc* test.

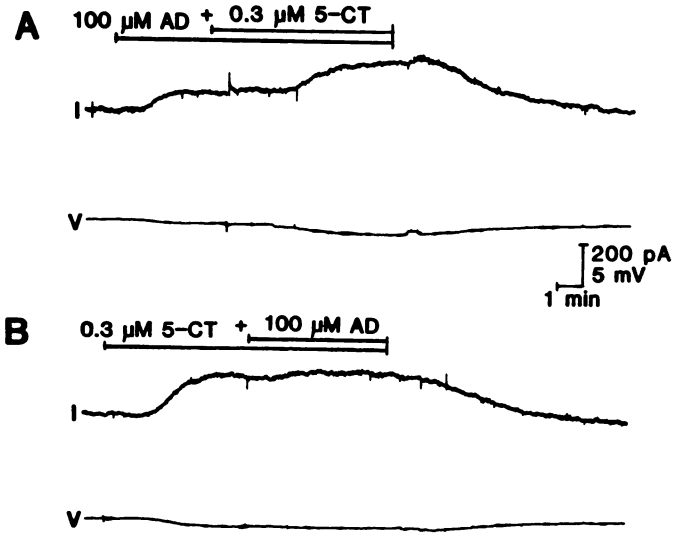

Fig. 8. Continuous voltage-clamp records of outward current evoked by 5-CT (0.3 μM) and/or AD (100 μM). The record tracings were obtained from the same cell clamped at its RMP of −70 mV, and the outward current evoked by agonist perfusion was quantified as described in Materials and Methods. Hippocampal slices were perfused with agonists for the length of time denoted by the *solid bar*. Tetrodotoxin (1 μM) was included in the ACSF in these experiments. A, Current (*I*)- voltage (*V*) record for 100 μM AD perfusion alone and with the subsequent addition of 0.3 μM 5-CT. B, Current-voltage record for 0.3 μM 5-CT perfusion alone and with the subsequent addition of 100 μM AD.

TABLE 3

Outward current measured in voltage-clamped hippocampal pyramidal cells in response to perfusion with 5-CT and/or AD

Hippocampal pyramidal cells were voltage-clamped at their RMP and the current elicited by application of 5-CT (0.3 μM) or AD (100 μM) was quantified; once steady state response was achieved, AD or 5-CT, respectively, was added to test for possible response additivity. Values were calculated as arithmetic mean ± standard error.

Agonist	*n*[a]	Current
		pA
5-CT	5	230 ± 17
AD	6	165 ± 15[b]
5-CT + AD	4	233 ± 23[c]
AD + 5-CT	5	286 ± 32[d]
Algebraic Sum		395[e]

[a] Number of cells.
[b] Value was significantly different ($p < 0.025$) from 5-CT value determined by unpaired *t* test.
[c] Value was significantly different ($p < 0.05$) from AD value determined by unpaired two-tailed *t* test.
[d] Value was significantly different ($p < 0.05$) from AD value determined by paired one-tailed *t* test. Paired *t* test excluded one AD value for which an AD + 5-CT value was not obtained.
[e] Value was significantly different from 5-CT + AD value ($p < 0.005$) and AD + 5-CT value ($p < 0.05$) determined by one sample *t* test.

3). No significant amount of additional current was obtained with the addition of AD after perfusion with 5-CT. An increase in current was observed with addition of 5-CT after AD perfusion; however, the total current was still significantly less than the expected current, calculated as their algebraic sum, if the receptors did not share any effector systems.

Discussion

In the present study, AD A_1 and 5-HT_{1A} receptors were demonstrated to coexist on the same hippocampal pyramidal cells and to activate two effector mechanisms: hyperpolarization of RMP, most probably mediated through enhanced K^+ conductance (2, 8–12),[3] and inhibition of adenylyl cyclase. Similar biochemical (4–7, 13–16) and electrophysiological (2, 8–12) results have been reported for AD and 5-HT agonists. The evidence presented here is consistent with the hypothesis that a common pool of PT-sensitive G proteins couple these receptors to the same effector systems (i.e., K^+ channels and adenylyl cyclase). These findings are supportive of prior studies that have demonstrated that AD A_1 (3, 31) and 5-HT_{1A} (2) receptors share signal-transducing components with other receptors (e.g., $GABA_B$ receptor). A similar coupling mechanism in which two receptor systems converge on the same conductance (e.g., K^+) has been reported for α_2-adrenergic receptors and opiate receptors in locus ceruleus neurons (32, 33) and $GABA_B$ and dopamine D_2 receptors in substantia nigra neurons (34).

The AD receptor mediating the inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes has pharmacological characteristics congruent with the AD A_1 binding site subtype (18, 19). The rank order of agonist EC_{50} values [(*R*)-PIA < NECA < PAA] and antagonist K_b values (CPT < theophylline < caffeine), as well as stereoselectivity [(*R*) PIA < (*S*)-PIA] are consistent with the classification of this receptor as the AD A_1 receptor. The spiperone-sensitive, 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase was characterized previously in this laboratory (7).

The EC_{50} values obtained for AD (Table 1) and 5-HT (7) agonists in functional studies are at least 10-fold greater than K_i values obtained for these agonists (19, 28) by competitive binding studies, with agonists retaining the same rank order of potency. A possible explanation for this apparent discrepancy is the two affinity states proposed for several receptor systems. Divalent cations such as Mg^{+2} and Mn^{+2} and guanine nucleotide analogs such as guanosine 5′-(β,γ-imido)triphosphate and guanosine-5′-*O*-(3-thio)triphosphate reciprocally modulate the two affinity states for a variety of binding sites related to functional receptors (35, 36). Divalent cations induce the high affinity state by promoting the formation of a transient ternary complex (37). Binding of agonists to the receptor, in the presence of guanine nucleotides, accelerates dissociation of the G protein complex from the receptor, causing the receptor to revert to the low affinity state. Previous studies have demonstrated that sites related to AD A_1 (38) and the 5-HT_{1A} (28, 39) receptors exist in these two interconvertible affinity states. Guanine nucleotides dextrally shift binding curves for AD A_1 and 5-HT_{1A} agonists approximately 1 order of magnitude. Results obtained from the linear regression (pK_i versus pEC_{50}) for AD and 5-HT agonists are consistent with the two affinity states suggested for these receptors. Significant correlations were obtained for both AD ($r = 0.98$) and 5-HT ($r = 0.85$) agonists, with slopes not statistically different ($p > 0.05$) from unity (Fig. 3). As would be expected, the EC_{50} values approximated the affinity (K_A) for the agonist for the low affinity state of the receptor.

The response parameters (EC_{50} and E_{max}) of an agonist are both drug- and tissue-dependent variables (40, 41). In the present study, (*R*)-PIA consistently produced larger E_{max} values for the inhibition of forskolin-stimulated adenylyl cyclase activity than did 5-HT (see Fig. 1). The difference in E_{max} values

[3] R. Greene, personal communication.

for (*R*)-PIA and 5-HT may reflect differences in receptor density and/or efficiency of receptor-effector coupling (i.e., intrinsic efficacy) (40, 41).

Previous electrophysiological studies have demonstrated that the hyperpolarizing response to 5-HT is mediated through the 5-HT_{1A} receptor (2, 11, 12). We show that the hyperpolarization produced by AD is mediated through the AD A_1 receptor subtype as evidenced by the response elicited by (*R*)-PIA, a selective AD A_1 agonist, and antagonism by CPT, a selective AD A_1 antagonist (Fig. 5A; Table 2). Previously the AD A_1 receptor was reported to elicit the decrease in amplitude of the extracellularly recorded field potential in CA1 area of hippocampal slices (42, 43).

The hyperpolarizations of RMP elicited by AD A_1 and 5-HT_{1A} agonists appear to be postsynaptic events because the addition of 1 μM tetrodotoxin in the ACSF did not affect this response (Fig. 8). Additionally, lowering extracellular Ca^{2+} concentrations to 200 μM and elevating extracellular Mg^{2+} concentrations to 4 mM in the superfusion medium, so as to block presynaptic, Ca^{2+}-dependent release, did not alter AD- and 5-HT-mediated hyperpolarizations (8, 9). Supportive of this hypothesis are quantitative autoradiographic studies demonstrating that very high densities of AD A_1 (44) and 5-HT_{1A} (45) binding sites are localized postsynaptically (46–48) in the CA1 region of the rat hippocampus. A smaller population (~30%) of AD A_1 binding sites have also been shown to be located presynaptically in this hippocampal region (46, 47); these presynaptic receptors have been implicated in the AD-mediated inhibition of neurotransmitter release (49). This differential localization of AD A_1 and 5-HT_{1A} binding sites may contribute to the greater E_{max} values exhibited by (*R*)-PIA (40%) relative to 5-HT (30%) in the biochemical studies (Fig. 1). It should be noted that this response parameter was measured in a broken cell preparation, which includes presynaptic, postsynaptic, and nonneuronal components. In contrast, the electrophysiological response was quantified in an intact, postsynaptic pyramidal cell, which may explain the similar reductions in RMP (~7 mV) elicited by both AD and 5-HT agonists (Table 2). For this reason, direct comparisons of biochemical response parameters cannot be made with those obtained from the electrophysiological studies.

The reduction in cAMP formation elicited by AD A_1 and 5-HT_{1A} agonists does not mediate the hyperpolarization of RMP produced by these drugs, because changes in intracellular cAMP levels failed to alter the hyperpolarizing effects evoked by activation of these receptor systems (2, 10). The hyperpolarization of the hippocampal pyramidal cell probably underlies the depression of neuronal excitability elicited by these agonists and may be the primary mechanism for the decrease in population spike amplitude elicited by AD (50, 51) and 5-HT (52, 53). Although the physiological role of the receptor-mediated inhibition of adenylyl cyclase has not been resolved (54), the decrease in intracellular cAMP accumulation might counteract the elevation in intracellular cAMP levels by receptors that stimulate adenylyl cyclase activity. For example, vasoactive intestinal peptide-stimulated cAMP accumulation is antagonized by 5-HT in cortical and striatal cells (55). Likewise, (*R*)-PIA has been shown to inhibit isoproterenol-stimulated adenylyl cyclase activity in cerebellar membranes (3). Alternatively, 5-HT_{1A} (56) and AD A_1 (57) agonists have been shown to reduce agonist-mediated phosphoinositide turnover in neuronal preparations.

Receptor-mediated inhibition of adenylyl cyclase activity and activation of K^+ channels are transduced through G proteins (35, 36). PT catalyzes the ADP-ribosylation of the α-subunit of G_i and G_o (58, 59), thereby inhibiting these receptor-mediated responses (35, 36). Previous studies demonstrated that PT reduced by 50–100% the inhibition of forskolin-stimulated adenylyl cyclase produced by (*R*)-PIA (15) and 5-HT (60). Likewise, the opening of K^+ channels by 5-HT (2) and AD (10) is transduced via a PT-sensitive G protein(s). In the present study, pretreatment with PT produced similar reductions in (*R*)-PIA- and 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes and hyperpolarization of RMP in the rat hippocampal slice preparation (Figs. 5B and 6; Table 2). However, the electrophysiological response was more sensitive to PT inactivation than was the biochemical response. For example, 4 μg of PT produced a 75% reduction of the hyperpolarization of RMP by 5-HT and (*R*)-PIA (Table 2) whereas only a 35% decrease in E_{max} values were observed with the same agonists in the biochemical studies (Fig. 6). Similar observations were reported previously in which PT pretreatment was more effective in reducing 5-HT_{1A} receptor-mediated decrease in population spike amplitude (~80%) than inhibition of forskolin-stimulated adenylyl cyclase activity (~50%), even though the two responses were significantly correlated (60). This difference in the magnitude of the effect of PT on the two responses may be related to the efficacy of coupling of these receptors to the two effector systems via the G protein(s). It is not known at this time whether the same G protein mediates both the inhibition of adenylyl cyclase and membrane hyperpolarization because PT inactivates at least three molecular forms of G_i (36), as well as G_o (59).

An intriguing finding was that the RMP of the pyramidal cells was unchanged after PT treatment (Table 2). Andrade *et al.* (2) previously reported no alterations in pyramidal cell characteristics following PT treatment. However, resting cell characteristics were altered in the presence of the AD A_1 receptor antagonist CPT in the present study (see below). A change in pyramidal cell RMP was expected, because PT blocks the coupling of receptors to G protein, which, in effect, inactivates the AD receptor. An explanation for this incongruity is not readily available.

The inclusion of CPT in the ACSF altered pyramidal cell resting membrane characteristics, resulting in a depolarization of the RMP and an increase in membrane resistance (Table 2). These data can be attributed to the antagonistic effect of CPT at AD A_1 receptors, resulting in the release of tonic inhibition by endogenous AD present in the synaptic cleft. Other groups have demonstrated a tonic inhibitory effect of AD, using a nonselective AD antagonist (caffeine), an AD uptake inhibitor (nitrobenzylthioinosine), and AD deaminase (61–64). Endogenous AD not only reduces cell excitability by hyperpolarizing the cell but also exerts a modulatory effect on other neurotransmitter systems (49). Supportive of this idea is the observation of a larger hyperpolarization elicited by 5-CT in the presence of CPT (Fig. 5; Table 2).

Both 5-HT and AD hyperpolarize hippocampal pyramidal cells (8, 9) primarily by increasing the K^+ conductance in these cells (2, 8–12).[3] Previously, the reversal potentials for 5-HT

and AD were reported to be about −90 mV (8, 9). In the present study, the lines in the current-voltage plot for 5-HT intersected at approximately −90 mV (Fig. 4C), indicative of a change in K^+ conductance. However, the lines for the AD current-voltage plots either did not intersect or did so at approximately −110 mV (Fig. 4C). Moreover, 5-HT elicited a greater change in membrane resistance than did AD, even though both agonists evoked equivalent hyperpolarization of RMP (Fig. 4; Table 2). These data implicate a localization of AD receptors distal to the cell body (62), where electrophysiological measurements were obtained.

Other evidence supportive of this hypothesis was derived from voltage-clamp experiments, which revealed a significant difference in the amount of outward current elicited by perfusion with a maximal concentration of 5-CT as compared with AD (Fig. 8; Table 3). Recently, Nicoll (31) also demonstrated that the current evoked by AD was 50% of the current elicited by 5-HT. These findings may be explained by the differential localization of 5-HT_{1A} and AD A_1 receptors on the same pyramidal cell. Autoradiographic studies have shown that AD receptors are located primarily on the distal dendrites of hippocampal pyramidal cells (44, 46, 47), whereas the 5-HT_{1A} receptors are distributed in all layers of the CA1 field of the hippocampus (45). Calculations using model systems have demonstrated that for conductance changes that occur at sites remote from the soma, voltage-clamp measurements may be reduced by 30% due to the electrotonic structure of the hippocampal neuron (65). Consequently, the current measured in response to AD perfusion may be underestimated by as much as 30%; the AD current measured in this study was 70% of the 5-CT current. Under current-clamp conditions, this disparity would be reflected in the differential changes in membrane resistance. Although there was a significant increase in the amount of additional outward current elicited when 5-CT and AD were coperfused, the total current produced was still much less than the expected current if 5-HT and AD receptors did not share effector mechanisms. Because there was no additional current evoked by perfusion of AD following 5-CT, we infer that AD A_1 receptors share a common pool of K^+ channels with a population of 5-HT_{1A} receptors, whereas a proportion of these receptors do not share K^+ channels with AD A_1 receptors.

Several lines of evidence suggest that a common pool of PT-sensitive G proteins couple AD A_1 and 5-HT_{1A} receptors to the same effector mechanisms. (i) AD A_1 and 5-HT_{1A} receptor-mediated hyperpolarizations of RMP were observed in the same hippocampal pyramidal cells, satisfying a neuroanatomical prerequisite for receptor colocalization (Fig. 4, A and B); (ii) PT administration caused similar reductions in (*R*)-PIA- and 5-HT-mediated inhibition of forskolin-stimulated adenylyl cyclase activity (ratio = 0.64–0.67) and hyperpolarization of RMP (Figs. 5B and 6; Table 2); and (iii) nonadditive responses to (*R*)-PIA and 5-HT were observed in both biochemical and electrophysiological studies, including agonist-mediated inhibition of adenylyl cyclase (Fig. 7) and hyperpolarization of RMP (Fig. 8; see preceding paragraph). It is highly unlikely that 5-HT agonists activate AD A_1 receptors and vice versa in the hippocampal preparations because high concentrations (>1 μM) of spiperone did not alter responses evoked by AD or (*R*)-PIA. Conversely, high concentrations (>1 μM) of CPT failed to antagonize biochemical or electrophysiological responses produced by 5-HT. Another mechanism that could account for the nonadditive responses obtained in the biochemical and electrophysiological studies is that AD A_1 and 5-HT_{1A} receptors share common G proteins that couple the receptors to the same effector systems. Although G proteins (G_i and G_o) constitute over 1% of all membrane proteins in brain (59), these coupling proteins may become rate limiting when a receptorial domain is activated by two agonists simultaneously (1). While G proteins are fundamental components of receptor-mediated signal transduction (35, 36), the sharing of G protein(s) by receptors coupled to the same effector systems may provide an auxiliary mechanism for the modulation of neuronal excitability.

Acknowledgments

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