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Lack of Apparent Receptor Reserve at Postsynaptic 5-Hydroxytryptamine$_{1A}$ Receptors Negatively Coupled to Adenylyl Cyclase Activity in Rat Hippocampal Membranes

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SUMMARY

Previous studies have demonstrated the existence of a large receptor reserve for agonists at somatodendritic 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) serotonin receptors in the raphe nuclei of the rat. 5-HT$_{1A}$ agonists with anxiolytic properties (e.g., buspirone, gepirone, and ipsapirone) display full intrinsic activity at these receptors but are partial agonists at postsynaptic 5-HT$_{1A}$ receptors, which suggests that the latter sites may be devoid of a receptor reserve. In the present studies, this was directly determined by examining the relationship between receptor occupancy and response at postsynaptic 5-HT$_{1A}$ receptors, in rat hippocampus, mediating the inhibition of forskolin-stimulated adenylyl cyclase activity, using the method of partial irreversible receptor inactivation. Rats were treated with vehicle or the irreversible antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), and 24 hr later hippocampi were removed for saturation analysis of [^{3}H]8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT) binding to 5-HT$_{1A}$ receptors or for adenylyl cyclase assays. EEDQ (1 and 6 mg/kg) dose-dependently reduced the maximal number of [^{3}H]8-OH-DPAT binding sites by 68.5 and 80%, respectively, without altering the K_d. Concentration-response curves were generated for inhibition of forskolin-stimulated adenylyl cyclase activity by 5-HT and the selective 5-HT$_{1A}$ agonist *N,N*-dipropyl-5-carboxamidotryptamine (DP-5-CT). EEDQ treatment dose-dependently reduced the maximal inhibitory effect of 5-HT [percentage of inhibition: control, 23.6; EEDQ (1 mg/kg), 13.4; EEDQ (6 mg/kg), 8.9], without altering either the slope factor (1.01) or the EC$_{50}$ (96.4 nM). Analogous results were obtained with DP-5-CT [percentage of maximal inhibition: control, 24.1; EEDQ (1 mg/kg), 15.2; EEDQ (6 mg/kg), 10.7), again without changes in slope factor (0.89) or EC$_{50}$ (9.9 nM). Analysis of double-reciprocal plots of equieffective concentrations of agonist, followed by calculation of fractional receptor occupancy, revealed a linear relationship between receptor occupancy and response for both 5-HT and DP-5-CT (i.e., an absence of receptor reserve). The receptor specificity of the effect of EEDQ was demonstrated in two ways. First, it was shown that pretreatment of rats with the selective 5-HT$_{1A}$ partial agonist BMY 7378 (10 mg/kg) before EEDQ afforded substantial protection (about 75%) against loss of the inhibitory effect of DP-5-CT on forskolin-stimulated adenylyl cyclase activity. Second, EEDQ did not alter the inhibition of forskolin-stimulated adenylyl cyclase activity induced by the adenosine A1 receptor agonist phenylisopropyladenosine (PIA). Because the inhibition of forskolin-stimulated adenylyl cyclase activity by PIA and 5-HT in the hippocampus appears to be mediated via a common pool of guanine nucleotide-binding regulatory protein(s) interacting with the same effector, EEDQ likely elicits its effects by inactivation of the 5-HT$_{1A}$ receptor active site and not by interference with intracellular proteins essential for signal transduction. The results support the hypothesis that the differential potency and efficacy of 5-HT$_{1A}$ agonists at pre- and postsynaptic 5-HT$_{1A}$ receptors reflects variation in the extent of receptor reserve at the two loci, rather than actions at different receptors.

The somewhat belated realization that nonbenzodiazepine anxiolytic drugs (e.g., buspirone and related azapirone analogs, such as gepirone and ipsapirone) are relatively selective agonists at 5-HT$_{1A}$ receptors (1, 2) has generated much interest in the anatomy, physiology, and function of this serotonin receptor subtype. These receptors are localized to the soma and/or dendrites of 5-HT neurons (autoreceptors) in the midbrain raphe nuclei and to postsynaptic targets in cortical and hippocampal regions, but not to 5-HT neuronal terminals (3, 4). The somatodendritic autoreceptors regulate local inhibitory control of neuronal firing (5, 6) and transmitter synthesis (7). Some

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; DP-5-CT, *N,N*-dipropyl-5-carboxamidotryptamine; EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid; PIA, phenylisopropyladenosine; BMY 7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4.5]-decane-7,9-dione dihydrochloride; G protein, guanine nucleotide-binding regulatory protein.

post-synaptic receptors (e.g., on hippocampal pyramidal cells) mediate a hyperpolarizing response by increasing a K^+ conductance (8), whereas others mediate inhibition of a forskolin-stimulated adenylyl cyclase, whose physiological function, however, is not yet known (9, 10).

The pre- and postsynaptic 5-HT$_{1A}$ receptors are also distinguished by their pharmacological properties. At somatodendritic autoreceptors, the prototypical agonist 8-OH-DPAT and the anxiolytic drugs elicit comparable degrees of maximal electrophysiological (6, 11, 12) and biochemical (13) response; in contrast, the latter agents appear to be partial agonists at postsynaptic 5-HT$_{1A}$ receptors, relative to the full or nearly full agonist 8-OH-DPAT (10, 12, 14).

We have previously demonstrated (13) the presence of a large receptor reserve for agonists at the somatodendritic 5-HT$_{1A}$ autoreceptor and proposed that this explains why 8-OH-DPAT and the anxiolytic drugs display equivalent intrinsic activities for agonist inhibition of 5-HT synthesis mediated by these autoreceptors. On the other hand, the partial agonist properties of buspirone and the other anxiolytics for eliciting functional responses mediated by postsynaptic 5-HT$_{1A}$ receptors (see above) suggest that there is little or no receptor reserve for agonists at these sites. It is relevant to note that an analogous pharmacological distinction of responses mediated by pre- and postsynaptic D2 dopamine receptors in the nigrostriatal system has been clarified as being due to the differential presence of a receptor reserve at pre- but not postsynaptic dopamine receptors (15, 16). In the present studies, we determined the extent of receptor reserve at the postsynaptic 5-HT$_{1A}$ receptor, in rat hippocampus, mediating inhibition of forskolin-stimulated adenylyl cyclase. As predicted, there appears to be no receptor reserve for this effect, and functional response is linearly related to receptor occupation.

Experimental Procedures

Materials. EEDQ, DP-5-CT, and PIA were purchased from Research Biochemicals, Inc. (Natick, MA). 5-HT, GTP, ATP, cAMP, EGTA, EDTA, dithiothreitol, phosphocreatine, creatine phosphokinase, and adenosine deaminase were obtained from Sigma Chemical Co. (St. Louis, MO). Forskolin was obtained from Calbiochem (San Diego, CA). [^{3}H]8-OH-DPAT (specific activity, 110 Ci/mmol) was obtained from Research Products International Corp. (Mt. Prospect, IL), whereas [α-^{32}P]ATP (specific activity, 30 Ci/mmol) and [^{3}H]cAMP (specific activity, 30.5 Ci/mmol) were from NEN Research Products (Wilmington, DE). All other reagents were of the highest chemical purity commercially available.

Drug treatments and tissue preparation. Male Sprague-Dawley rats (180–280 g; Harlan Labs, Indianapolis, IN) were treated with EEDQ (1 or 6 mg/kg, subcutaneously) or vehicle 24 hr before sacrifice. In some cases, rats were treated with BMY 7378 (10 mg/kg, subcutaneously) 30 min before EEDQ or vehicle. Animals were sacrificed by decapitation, and the entire hippocampus was dissected on ice and either stored at −80° for receptor binding assays or homogenized immediately for adenylyl cyclase assays. Tissues were homogenized (Teflon-glass) in STEED buffer containing 0.32 M sucrose, 20 mM Tris·HCl, 5 mM EDTA, 1 mM EGTA, and 5 mM dithiothreitol, pH 7.4, further diluted 8-fold in STEED buffer, and centrifuged at 39,000 × g for 10 min at 0°. The supernatant was discarded, and the pellet was reconstituted in 40 volumes of cold STEED buffer and stored frozen at −80° until used in the adenylyl cyclase assay.

Adenylyl cyclase assay. Inhibition of forskolin-stimulated adenylyl cyclase by activation of 5-HT$_{1A}$ and adenosine A1 receptors was carried out as described by Zgombick *et al.* (17). Aliquots (50 μl) of membrane homogenate were added to tubes containing 200 μl of reaction mixture (pH 7.4), which had been preincubated at 30° for 5 min. The reaction mixture consisted of (in mM) forskolin, 0.01; Tris·HCl, 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; plus 2 units/ml adenosine deaminase, 1 μCi of [α-^{32}P]ATP, and various concentrations of drugs. The tubes were incubated for an additional 5 min at 30°, and the reaction was terminated by addition of 100 μl of 2% (w/v) sodium dodecyl sulfate. After the addition of approximately 20,000 cpm of [^{3}H]cAMP, to monitor the recovery of [^{32}P]cAMP, the tubes were placed in a boiling water bath for 5 min. The tubes were allowed to cool for 1 hr, and cAMP was purified by sequential elution from Dowex AG 50W-X4 (200–400 mesh) and alumina (activity grade 1, type WN-3 neutral) columns. Radioactivity was measured by liquid scintillation counting.

[^{3}H]8-OH-DPAT binding assay. Saturation binding studies were performed using a modification of the method of Hall *et al.* (18). Hippocampal tissues were homogenized in cold buffer (50 mM Tris·HCl, 2 mM EGTA, pH 7.4) with a Brinkmann Polytron. Additional buffer was added (total volume, 40 ml), and the homogenates were centrifuged at 39,000 × g for 10 min. The pellet was resuspended and centrifuged again, and the second pellet was resuspended in 40 ml of assay buffer (50 mM Tris·HCl, 5 mM $MgSO_4$, 2 mM EGTA, 10 μM pargyline, 0.01% ascorbic acid, pH 7.7) and incubated at 37° for 10 min, to remove endogenous 5-HT (19). After centrifugation, the final pellet was resuspended in assay buffer (100 volumes), and 0.8 ml of the suspension was distributed to assay tubes. After addition of 0.1 ml of [^{3}H]8-OH-DPAT (12 concentrations, 0.1–10 nM) and 0.1 ml of buffer or 10 μM 5-HT, to define nonspecific binding, the tubes were incubated for 10 min at 37°, followed by rapid filtration (Brandel cell harvester) over Whatman GF/B filters. The filters were washed three times (50 mM Tris·HCl, pH 7.4), and radioactivity on the filters was measured by liquid scintillation counting. Binding data were analyzed with the RS/1 data analysis software package (BBN Software Products, Cambridge, MA).

Data analysis. Concentration-response curves for 5-HT and DP-5-CT inhibition of forskolin-stimulated adenylyl cyclase in hippocampus of vehicle- and EEDQ-treated rats were simultaneously analyzed for best fit, using the ALLFIT program (20), as described previously in detail (13, 15). In brief, curves were analyzed without initial constraints and with successive constraint of the fits to share a common slope factor, EC_{50}, and maximal response. Minimal response (response at zero drug concentration) was set to a constant (100% of control) for all curves. The analysis that permitted the largest number of shared constraints without a significant degradation in fit was chosen as the best fit. The agonist dissociation constants (K_A) for 5-HT and DP-5-CT inhibition of forskolin-stimulated adenylyl cyclase were obtained by the method of Furchgott and Bursztyn (21), using the equation

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{1-q}{qK_A}$$

where $[A]$ is the concentration of agonist necessary to produce a particular level of response before inactivation, $[A']$ is the concentration needed to produce the same response after inactivation, and q is the fraction of remaining intact receptors. K_A values were obtained by plotting the reciprocals of the equieffective concentrations of agonist after inactivation, $1/[A']$, against the reciprocals of the concentrations before inactivation, $1/[A]$, for each pair of concentration-response curves. The equieffective concentrations were determined at five levels of response (30, 40, 50, 60, and 70% of the maximum effect after EEDQ treatment) (13, 15), from the ALLFIT-derived best-fit concentration-response curves. Each resulting straight line had a slope of $1/q$ and K_A equal to (slope − 1)/y-intercept.

The K_A values were used to calculate fractional receptor occupancy (f) at a particular concentration $[A]$, from the law of mass action:

$$f = [RA]/[R_T] = [A]/K_A + [A]$$

where $[RA]$ is the concentration of receptor-agonist complex and $[R_T]$ is the initial (total) concentration of active receptors. Fractional receptor occupancy at a particular concentration was then plotted against fractional response at that concentration (obtained from the control best-fit concentration-response curve).

Results

Effects of EEDQ on forskolin-stimulated adenylyl cyclase. EEDQ treatment (1 or 6 mg/kg) did not significantly modify basal adenylyl cyclase activity in hippocampal membranes [pmol/mg of tissue/min, mean ± standard error (number of experiments): vehicle, 1.08 ± 0.09 (3); 1 mg/kg EEDQ, 1.03 ± 0.07 (4); 6 mg/kg EEDQ, 1.01 ± 0.04 (4)]. Forskolin (10 μM)-stimulated adenylyl cyclase was likewise unaltered by EEDQ treatment [pmol/mg of tissue/min, mean ± standard error (number of experiments): vehicle, 6.01 ± 0.16 (3); 1 mg/kg EEDQ, 6.52 ± 0.34 (4); 6 mg/kg EEDQ, 6.04 ± 0.32 (4)].

Effects of EEDQ on [^{3}H]8-OH-DPAT binding to 5-HT$_{1A}$ receptors in rat hippocampal membranes. Treatment of rats with 1 and 6 mg/kg EEDQ resulted in a dose-dependent reduction in the maximal number of binding sites (B_{max}) for [^{3}H]8-OH-DPAT, without altering the affinity of the ligand (K_d) (Table 1). EEDQ treatment (1 and 6 mg/kg) reduced the maximal number of binding sites to 31.5 and 20% of control, respectively.

Effects of partial irreversible receptor inactivation by EEDQ on inhibition of forskolin-stimulated adenylyl cyclase by 5-HT and DP-5-CT. In vehicle-pretreated rats, 5-HT maximally inhibited forskolin-stimulated adenylyl cyclase in hippocampal membranes by 23.6%, with an EC_{50} of 96.4 nM (Fig. 1). In membranes taken from rats treated 24 hr earlier with EEDQ (1 and 6 mg/kg), simultaneous ALLFIT analysis indicated that the slope factor (1.01) and EC_{50} (96.4 nM) could be shared without a significant worsening of the fit; constraining the curves to share a common maximal response, however, resulted in a much poorer fit ($p < 0.001$). The ALLFIT-derived maxima after 1 and 6 mg/kg EEDQ were 13.4 and 8.9% (corresponding to 57 and 38% of the maximal response obtained in vehicle controls), respectively. Qualitatively analogous results were obtained when the selective full 5-HT$_{1A}$ agonist DP-5-CT was used (Fig. 2). Maximal inhibition was reduced from 24.1% in controls to 15.2 and 10.7% after 1 and 6 mg/kg EEDQ, respectively (corresponding to 63 and 44% of the maximal vehicle response, respectively). Neither the slope factor (0.89) nor the EC_{50} (9.9 nM) was altered by receptor inactivation. It should be noted that each dose of EEDQ had a more marked effect in reducing the binding of [^{3}H]8-OH-DPAT than in reducing the maximal functional response (see Discussion). These results, revealing a dose-dependent reduction in

TABLE 1

Effects of EEDQ on [^{3}H]8-OH-DPAT saturation binding parameters

Binding of [^{3}H]8-OH-DPAT to hippocampal membranes was carried out as described in Experimental Procedures. Each value is the mean ± standard error of four determinations.

Treatment	K_d	B_{max}
	nM	*fmol/mg of protein*
Vehicle	1.52 ± 0.04	232 ± 7
EEDQ, 1 mg/kg	1.57 ± 0.08	73 ± 2 (−68.5%)
EEDQ, 6 mg/kg	1.70 ± 0.23	37 ± 2 (−80%)

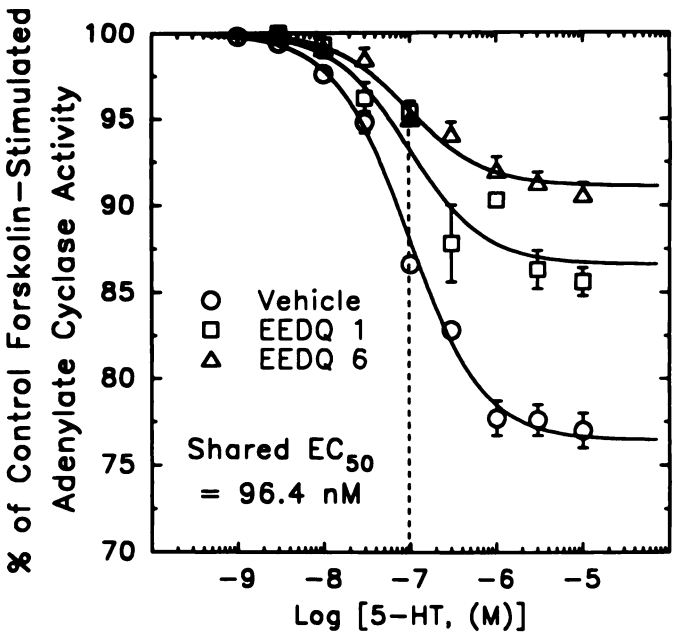

Fig. 1. Concentration-response curves for 5-HT inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampal membranes. Best-fit curves (*solid lines*) are derived from simultaneous ALLFIT analysis in which all three data sets, when constrained to a constant minimum response (100% of control) and shared slope factor and EC_{50}, did not differ significantly ($p > 0.05$) from other fits in which fewer parameters were constrained to be shared. Significant ($p < 0.001$) differences in maximal response were obtained, however (57 and 38%, relative to vehicle control, after 1 and 6 mg/kg EEDQ, respectively). In this and all subsequent figures, each *point* is the mean ± standard error of three separate determinations.

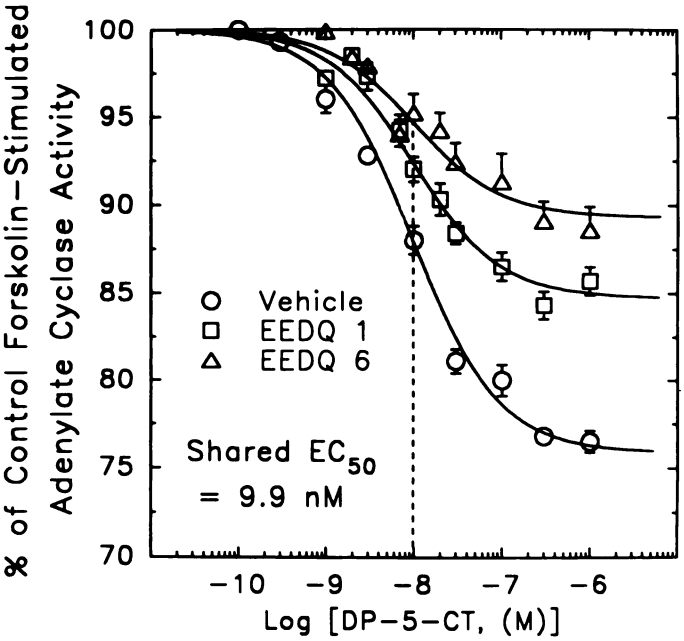

Fig. 2. Concentration-response curves for DP-5-CT inhibition of forskolin-stimulated adenylyl cyclase activity. The data were subjected to the same constraints as described in the legend to Fig. 1. Maximal responses after 1 and 6 mg/kg EEDQ were 63 and 44%, respectively.

maximal response without an alteration in the EC_{50}, are consistent with a postulated absence of receptor reserve (16, 22).

Relationship between receptor occupancy and response. Concentration-response curves obtained for each agonist in EEDQ- and vehicle-treated rats were subjected to analysis by the double-reciprocal method of Furchgott and Bursztyn (21). Equieffective agonist concentrations of 5-HT and DP-5-CT needed to elicit a series of identical levels of response in control and EEDQ-treated rats were plotted and are shown in Figs. 3 and 4. The q values (fraction of remaining active receptors after EEDQ treatment) obtained are in very good agreement with the level of maximal response obtained after each corresponding dose of EEDQ (see above). Furthermore, for each agonist, the calculated dissociation constant (K_A) (concentration required to occupy 50% of the receptors) was essentially identical, regardless of which EEDQ curve (1 or 6 mg/kg) was used in its determination. Because the K_A values are very similar to the corresponding EC_{50} values for each agonist (Figs. 1 and 2), it is apparent that half-maximal response occurs at approximately half-maximal receptor occupancy, i.e., the relationship between receptor occupancy and response is linear. This is evident in Fig. 5, where the K_A values obtained were used to calculate fractional receptor occupancy for each concentration of agonist and were plotted against fractional response obtained at that concentration. The results reveal that there is a linear relationship, for both 5-HT and DP-5-CT, between occupancy and response at postsynaptic rat hippocampal 5-HT$_{1A}$ receptors negatively coupled to adenylyl cyclase and, therefore, an absence of receptor reserve. Half-maximal response occurred at 50.2 and 47.7% receptor occupancy for 5-HT and DP-5-CT, respectively. In contrast, a steep hyperbolic relationship, indicative of a large receptor reserve, was obtained previously (13) for the full agonist 8-OH-DPAT

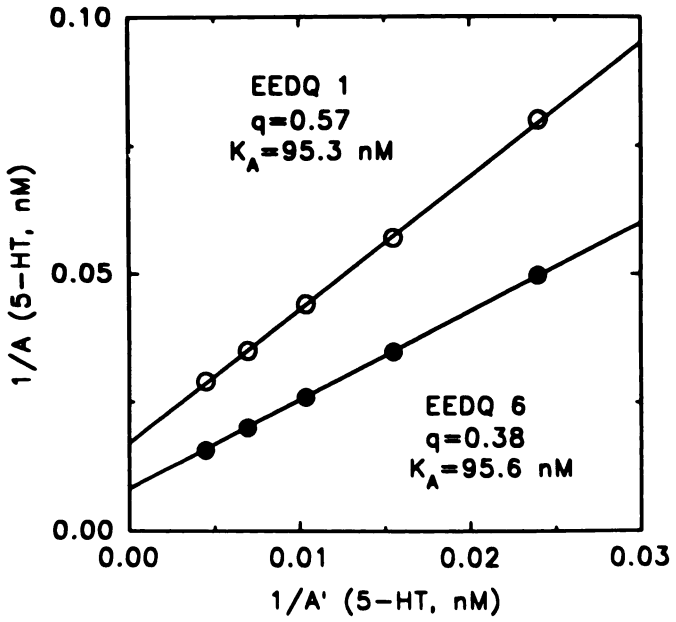

Fig. 3. Double-reciprocal plots of equieffective concentrations of 5-HT required to elicit response at five levels of effect (30–70% of the maximal response) in paired vehicle and EEDQ curves at each dose of EEDQ. Note that q values (fraction of remaining active receptors) were very similar to fractional maximal response after each EEDQ dose; likewise, K_A values agreed well not only for each dose of EEDQ but also with the ALLFIT-derived EC_{50} value (Fig. 1).

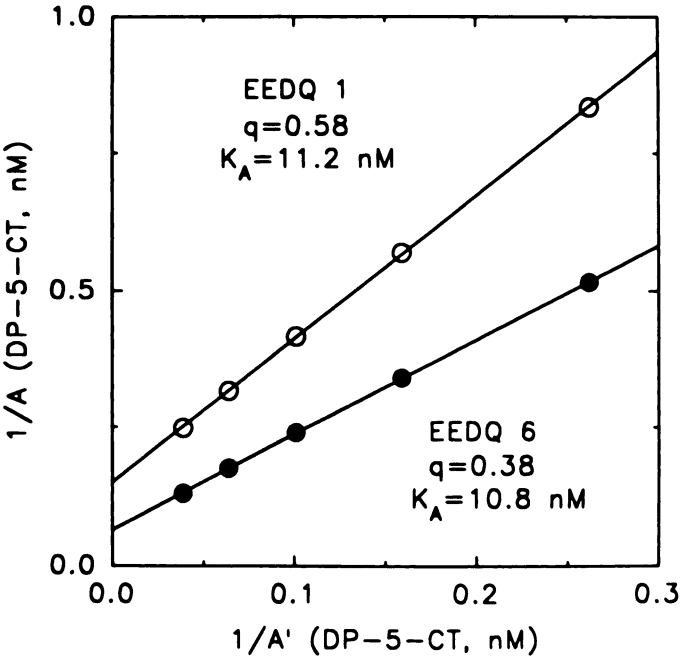

Fig. 4. Double-reciprocal plots for DP-5-CT. As for the 5-HT data, q values agreed well with fractional maximal response after each dose of EEDQ, and calculated K_A values were close to the ALLFIT-derived EC_{50} (Fig. 2).

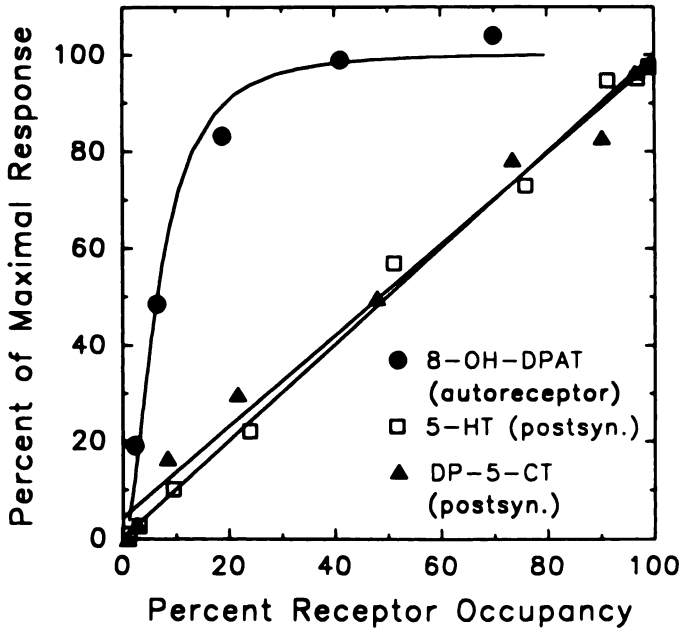

Fig. 5. Relationship between receptor occupancy and response for 5-HT and DP-5-CT inhibition of forskolin-stimulated adenylyl cyclase activity. Fractional receptor occupancy (f) for the agonists was calculated from the law of mass action ($f = [A]/K_A + [A]$), using the average K_A values obtained in the double-reciprocal plots (Figs. 3 and 4) and the corresponding ALLFIT-derived vehicle concentration-response curves (Figs. 1 and 2), as described in Experimental Procedures. The data points for 5-HT and DP-5-CT were subjected to least squares regression analysis. The data for *in vivo* 8-OH-DPAT inhibition of 5-hydroxytryptophan accumulation mediated by somatodendritic 5-HT$_{1A}$ autoreceptors are taken from Ref. 13.

at somatodendritic 5-HT_{1A} autoreceptors controlling the synthesis of 5-HT in rat hippocampus, as shown for comparison.

Specificity of the effect of EEDQ at 5-HT_{1A} receptors mediating inhibition of forskolin-stimulated adenylyl cyclase. EEDQ irreversibly inactivates subtypes of dopamine, α-adrenergic, and, at higher doses, muscarinic (23) and benzodiazepine (24) receptors, in addition to 5-HT receptors. Furthermore, EEDQ also has the potential (although usually rather low; see Refs. 25 and 26) to inactivate nonspecifically intracellular proteins (e.g., G proteins and/or effectors) that are crucial to the generation of the functional response of interest. Wherever possible, therefore, it is highly desirable to establish that the effects of EEDQ in a particular functional assay are restricted to inactivation of the receptor site in question. Previously, we demonstrated (13) that selective protection of somatodendritic 5-HT_{1A} autoreceptors by pretreatment with the partial agonist BMY 7378 (27) prevented the EEDQ-induced loss of inhibition of 5-hydroxytryptophan accumulation produced by either 8-OH-DPAT or buspirone. In a similar fashion, occlusion of 5-HT_{1A} receptors by treatment with BMY 7378 (10 mg/kg) before EEDQ (6 mg/kg) resulted in a significant protection (about 75%) of the ability of DP-5-CT to inhibit forskolin-stimulated adenylyl cyclase activity (Fig. 6). BMY 7378 pretreatment alone did not significantly alter the concentration-response curve for DP-5-CT. These results suggest that the effect of EEDQ in reducing the ability of the agonists to inhibit forskolin-stimulated adenylyl cyclase activity is due to the specific covalent modification of the 5-HT_{1A} receptor binding site.

Additional evidence for the hypothesis described above was obtained by examining the effect of EEDQ on the PIA-induced inhibition (via activation of adenosine A1 receptors) of forskolin-stimulated adenylyl cyclase. The inhibitory effects of both PIA and 5-HT on this enzyme in the hippocampus appear to be mediated by a common pool of G protein(s) acting through a common effector (18). EEDQ treatment (6 mg/kg) did not significantly affect PIA-induced inhibition of forskolin-stimulated adenylyl cyclase activity (Fig. 7). Although the effects of EEDQ on adenosine A1 receptor binding were not determined, these results suggest that EEDQ does not inactivate these receptors. Furthermore, because the functional response to PIA was not altered, it may be inferred that *in vivo* EEDQ treatment does not alter either the G protein(s) or effector moieties that couple to adenosine A1 and 5-HT_{1A} receptors and mediate the inhibition of forskolin-stimulated adenylyl cyclase activity (18).

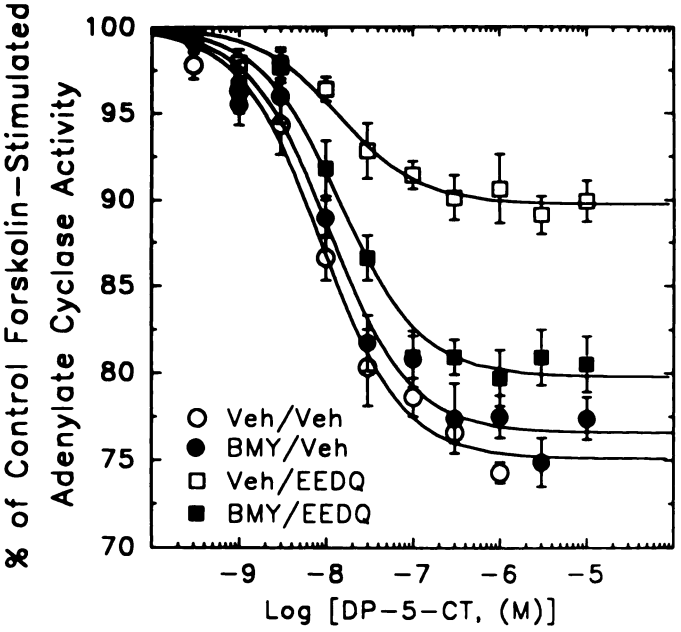

Fig. 6. Protection of DP-5-CT inhibition of forskolin-stimulated adenylyl cyclase activity by pretreatment with BMY 7378. All four curves were analyzed simultaneously by ALLFIT. The *solid lines* show the data conservatively fit to a constant minimum response (100% of control) and a shared slope factor (0.89), which yielded small variations in EC_{50} (range, 8.7–14.3 nM) and differences in maximal response (range, 10.3–24.9% inhibition). The data could be further constrained to share a common EC_{50} (10.7 nM), and the vehicle/vehicle and BMY/vehicle curves could be constrained to share a common maximum (24.2% inhibition) without a significant increase in the residual variance for the fit ($p > 0.05$). The protection afforded by BMY 7378 (about 75%) was not complete, however, because the BMY/EEDQ curve maximum was significantly different from that of the vehicle/vehicle and BMY/vehicle curves ($p < 0.001$). *Veh*, vehicle.

Discussion

From the data presented above, there is no apparent receptor reserve for 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampus. This conclusion derives from the observation that for both agonists, after two different levels of receptor inactivation (1 and 6 mg/kg EEDQ), the calculated agonist dissociation constants were in excellent agreement with the experimental EC_{50} values (5-HT: EC_{50}, 96.4 nM; K_A, 95.3 and 95.6 nM, respectively; DP-5-CT: EC_{50}, 9.9 nM; K_A, 11.2 and 10.8 nM, respectively). Thus, the concentration of each agonist that elicited half-maximal

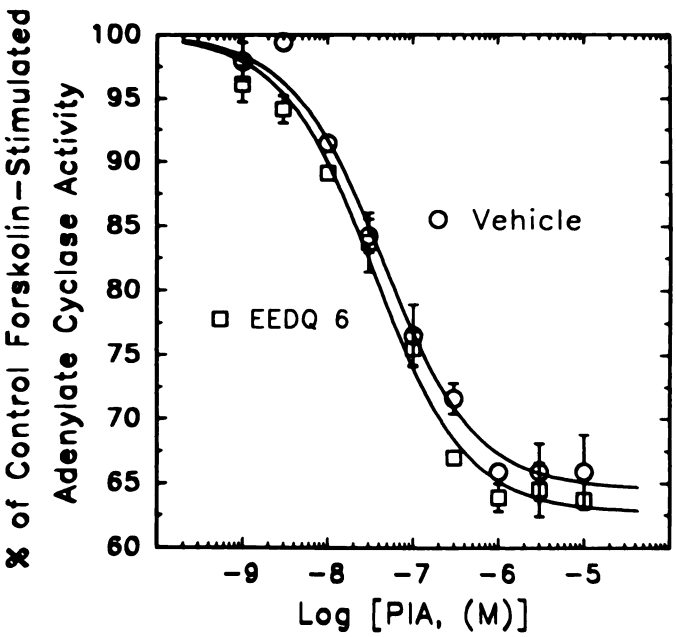

Fig. 7. Effect of EEDQ on PIA inhibition of forskolin-stimulated adenylyl cyclase activity. For pictorial reasons, the curve fits shown are the result of constraint to a constant minimum response at zero concentration (100% of control) and a shared slope factor (0.79). However, ALLFIT analysis indicated that the two curves could share all four parameters (minimum and maximum response, slope factor, and EC_{50}) without a significant degradation in fit (i.e., all the points could be considered to belong to the same data set) (slope factor, 0.80; EC_{50}, 38.9 nM; maximum, 36.3% inhibition).

response was indistinguishable from that required to occupy 50% of the functionally relevant receptors. Furthermore, there was also very good agreement between the percentage of control maximal effect (E_{max}) attained after receptor inactivation and the calculated percentage ($q \times 100$) of remaining active receptors [5-HT: E_{max}, 57 and 38%; $q \times 100$, 57 and 38% (1 and 6 mg/kg EEDQ, respectively); DP-5-CT: E_{max}, 63 and 44%; $q \times 100$, 58 and 38% (1 and 6 mg/kg EEDQ, respectively)]. Both of these features are hallmarks of the absence of receptor reserve (22). Taken together with our previous findings of a large receptor reserve for somatodendritic 5-HT$_{1A}$ autoreceptor-mediated inhibition of serotonin synthesis (13), the results support the hypothesis that the differential potency and efficacy of various agonists to elicit responses mediated by these receptors reflect differences in the efficiency of receptor/effector coupling (i.e., receptor reserve) at these loci. Partial agonists can display full intrinsic activity (maximal apparent response) in the presence of a receptor reserve (13, 21, 22). Thus, 5-HT$_{1A}$ agonists such as buspirone, gepirone, and ipsapirone are able to inhibit potently and completely the firing of 5-HT neurons in the raphe nuclei (5, 6, 11, 12) after systemic or iontophoretic administration, similar to the effects of 5-HT and the full agonist 8-OH-DPAT, but display partial agonist properties at postsynaptic sites in the hippocampus (12, 28). The inhibition of neuronal firing by serotonin appears to be mediated by the activation of a pertussis toxin-sensitive K^+ conductance in both brain regions (29–31). Because buspirone, gepirone, and ipsapirone act as partial agonists for opening K^+ channels in the hippocampus (29), it appears likely that there is a difference in the extent of receptor reserve between the dorsal raphe and the hippocampus, even for this identical membrane electrical effect. Similarly, the ability of these agonists to inhibit maximally and equivalently autoreceptor-mediated 5-HT synthesis (13), but to exhibit partial agonist effects on forskolin-stimulated adenylyl cyclase activity in the hippocampus (10), is also consistent with a differential receptor reserve.

It is also of interest to note that certain behavioral effects of 5-HT$_{1A}$ agonists (elicitation of components of the serotonin syndrome) appear to be mediated via activation of postsynaptic 5-HT$_{1A}$ receptors (32). Although the precise anatomic locus of the receptors responsible for the behavioral effects, or the signal transduction mechanism(s) they are coupled to, is not known, it has been shown that buspirone, gepirone, and ipsapirone are partial agonists for elicitation of the serotonin syndrome (33, 34). It may, therefore, be inferred that, regardless of the second messenger system(s) utilized to effect the behavioral responses, it is likely that the relevant receptors exhibit little or no receptor reserve for agonists.

The discrepancy between the extent of reduction of 5-HT$_{1A}$ receptor binding sites labeled by [^{3}H]8-OH-DPAT and the percentage of reduction in maximal functional response after EEDQ treatment deserves some comment. EEDQ reduced the B_{max} for [^{3}H]8-OH-DPAT to 31.5 and 20% of control after 1 and 6 mg/kg, respectively (Table 1). In contrast, these same doses of EEDQ reduced the maximal response for 5-HT and DP-5-CT inhibition of forskolin-stimulated adenylyl cyclase activity to 57–63 and 38–44% of control, respectively (Figs. 1 and 2). *A priori*, a comparison of the binding and functional data would suggest that there is some degree of nonlinearity between occupancy and response for inhibition of forskolin-stimulated adenylyl cyclase activity, because 57–63% of maximal functional response can be obtained in the presence of 31.5% of the total complement of receptors. Likewise, 38–44% of the maximal response can be obtained with only 20% of the receptor binding sites. Interestingly, Creese and colleagues (25, 35) have compared EEDQ-induced loss of D1 dopamine receptor binding sites and D1-stimulated adenylyl cyclase in rat striatum and found a similar discrepancy, a greater reduction in binding sites than in functional response at each dose of EEDQ. They concluded that this provided evidence for spare receptors, despite the fact that, as in our data described above, there appeared to be no significant alteration in the EC_{50} for the agonist (35), which would be expected in the presence of a receptor reserve (22). We previously investigated this issue and corroborated the lack of a shift in EC_{50} for D1-stimulated adenylyl cyclase activity (36); furthermore, analysis of those data by the Furchgott method (rather than by comparison with changes in receptor binding density) indicated the absence of receptor reserve for this response (36). It has been pointed out by Kenakin (37) that there are inherent potential errors introduced by directly comparing changes in radioligand receptor binding density with changes in functional response. Alternatively, there may be either a small receptor reserve or small deviations from linearity in the relationship between receptor occupancy and response, whose importance tends to be reduced by the curve-fitting analyses because small differences in EC_{50} are statistically nonsignificant. Another possibility is that, because not all 5-HT$_{1A}$ receptors in the hippocampus are coupled to adenylyl cyclase, a preferential inactivation by EEDQ of non-cyclase-linked receptors would result in a larger reduction in B_{max} than in E_{max}. However, we have no evidence for such an effect.

It is worth pointing out here that both somatodendritic and postsynaptic 5-HT$_{1A}$ receptors are coupled to their effectors via pertussis-toxin sensitive G proteins (38, 39). It is highly likely that differences in receptor reserve reflect mechanistic differences in one or more factors crucial to efficient signal transduction, including (but not limited to) 1) the nature of the G protein and its coupling affinity for the receptor; 2) the stochiometric relationship between receptor, G protein, and effector; and 3) the nature of the effector. We have recently shown that, in systems displaying a large receptor reserve for agonists, partial G protein inactivation with pertussis toxin produced changes in agonist dose-response curves that were analogous to those brought about by partial receptor inactivation (40). That is, for full agonists the dose-response curves were substantially shifted to the right, with only small reductions in maximal effect (i.e., there appeared to be a "G protein reserve"). Thus, receptor/G protein stochiometry appears to be an important factor in determining receptor/effector coupling efficiency. It would be reasonable to predict, therefore, that in systems devoid of receptor reserve (as is apparently the case for 5-HT$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase activity) there should also be little G protein reserve. That this is indeed the case is borne out by the work of Clarke *et al.* (39), who previously demonstrated that pertussis toxin reduced the maximal response for 5-HT inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampus without alteration in the EC_{50}. Together with the present data, this supports the idea that there is a correlation between receptor reserve and G protein reserve (40).

In summary, we have demonstrated here that there is little

or no apparent receptor reserve for 5-HT_{1A} receptor-mediated inhibition of forskolin-stimulated adenylyl cyclase activity in rat hippocampus, whereas, in contrast, earlier work established the existence of a large receptor reserve at the somatodendritic 5-HT_{1A} autoreceptor in the raphe nuclei. Collectively, the results appear to explain the differences in potency and efficacy of various agonists at these sites to elicit functional responses.

References

1. Peroutka, S. J. Selective interaction of novel anxiolytics with 5-hydroxytryptamine$_{1A}$ receptors. *Biol. Psychiatry* **20**:971–979 (1985).
2. Traber, J., and T. Glaser. 5-HT_{1A} receptor-related anxiolytics. *Trends Pharmacol. Sci.* **8**:432–437 (1987).
3. Weissmann-Nanopoulos, D., E. Mach, J. Magre, Y. Demassey, and J.-F. Pujol. Evidence for the localization of 5-HT1A binding sites on serotonin containing neurons in the raphe dorsalis and raphe centralis nuclei of the rat brain. *Neurochem. Int.* **7**:1061–1072 (1985).
4. Verge, D., G. Daval, A. Patey, H. Gozlan, S. El Mestikawy, and M. Hamon. Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT_{1A} subtype. *Eur. J. Pharmacol.* **113**:463–464 (1985).
5. VanderMaelen, C. P., G. K. Matheson, R. C. Wilderman, and L. A. Patterson. Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration of buspirone, a non-benzodiazepine anxiolytic drug. *Eur. J. Pharmacol.* **129**:123–130 (1986).
6. Sprouse, J. S., and G. K. Aghajanian. Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT_{1A} and 5-HT_{1B} agonists. *Synapse* **1**:3–9 (1987).
7. Hjorth, S., and T. Magnusson. The 5-HT_{1A} receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain *in vivo*. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **338**:463–471 (1988).
8. Colino, A., and J. V. Halliwell. Differential modulation of three separate K-conductances in hippocampal CA1 neurons by serotonin. *Nature* (*Lond.*) **328**:73–77 (1987).
9. De Vivo, M., and S. Maayani. Characterization of the 5-hydroxytryptamine$_{1A}$ receptor-mediated inhibition of forskolin-stimulated adenylate cyclase activity in guinea pig and rat hippocampus. *J. Pharmacol. Exp. Ther.* **238**:248–253 (1986).
10. Bockaert, J., A. Dumuis, R. Bouhelal, M. Sebben, and R. N. Cory. Piperazine derivatives including the putative anxiolytic drugs, buspirone and ipsapirone, are agonists at 5-HT_{1A} receptors negatively coupled with adenylate cyclase in hippocampal neurons. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **335**:588–592 (1987).
11. Sinton, C. M., and S. L. Fallon. Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT_1 receptor. *Eur. J. Pharmacol.* **157**:173–181 (1988).
12. Sprouse, J. S., and G. K. Aghajanian. Responses of hippocampal pyramidal cells to putative serotonin 5-HT_{1A} and 5-HT_{1B} agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology* **7**:707–715 (1988).
13. Meller, E., M. Goldstein, and K. Bohmaker. Receptor reserve for 5-hydroxytryptamine$_{1A}$-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine$_{1A}$ agonists. *Mol. Pharmacol.* **37**:231–237 (1990).
14. Martin, K. F., and R. Mason. Ipsapirone is a partial agonist at 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors in the rat hippocampus: electrophysiological evidence. *Eur. J. Pharmacol.* **141**:479–483 (1987).
15. Meller, E., K. Bohmaker, Y. Namba, A. J. Friedhoff, and M. Goldstein. Relationship between receptor occupancy and response at striatal dopamine autoreceptors. *Mol. Pharmacol.* **31**:592–598 (1987).
16. Meller, E., A. Enz, and M. Goldstein. Absence of receptor reserve at striatal dopamine receptors regulating cholinergic activity. *Eur. J. Pharmacol.* **155**:151–154 (1988).
17. Zgombick, J. M., S. G. Beck, C. D. Mahle, B. Craddock-Royal, and S. Maayani. Pertussis toxin-sensitive guanine nucleotide-binding protein(s) couple adenosine A1 and 5-hydroxytryptamine$_{1A}$ receptors to the same effector systems in rat hippocampus: biochemical and electrophysiological studies. *Mol. Pharmacol.* **35**:484–494 (1989).
18. Hall, M. D., S. El-Mestikawy, M. B. Emerit, L. Pichat, M. Hamon, and H. Gozlan. [^{3}H]8-Hydroxy-2-(di-*n*-propylamino)tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J. Neurochem.* **44**:1685–1696 (1985).
19. Nelson, D. L., A. Herbert, S. Bourgoin, J. Glowinski, and M. Hamon. Characterization of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine in the rat. *Mol. Pharmacol.* **14**:983–995 (1978).
20. De Lean, A., P. Munson, and D. Rodbard. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose response curves. *Am. J. Physiol.* **235**:E97–E102 (1978).
21. Furchgott, R. F., and P. Bursztyn. Comparison of dissociation constants and of relative efficacies of selected agonists acting on parasympathetic receptors. *Ann. N. Y. Acad. Sci.* **144**:882–899 (1967).
22. Ruffolo, R. R. Important concepts of receptor theory. *J. Autonom. Pharmacol.* **2**:277–295 (1982).
23. Norman, A. B., and I. Creese. Effects of *in vivo* and *in vitro* treatments with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline on putative muscarinic receptor subtypes in rat brain. *Mol. Pharmacol.* **30**:96–103 (1986).
24. Miller, L. G., M. Lumpkin, D. J. Greenblatt, and R. I. Shader. Accelerated benzodiazepine receptor recovery after lorazepam discontinuation. *FASEB J.* **5**:93–97 (1991).
25. Hess, E. J., G. Battaglia, A. B. Norman, and I. Creese. Differential modification of striatal D1 dopamine receptors and effector moieties by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline *in vivo* and *in vitro*. *Mol. Pharmacol.* **31**:50–57 (1987).
26. Norman, A. B., T. J. Wachendorf, and P. R. Sanberg. Differential effect of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) on [^{3}H]SCH 23390 and [^{3}H]forskolin binding in rat striatum. *Life Sci.* **44**:831–836 (1989).
27. Yocca, F. D., D. K. Hyslop, D. W. Smith, and S. Maayani. BMY 7378, a buspirone analog with high affinity, selectivity and low intrinsic activity at the 5-HT_{1A} receptor in rat and guinea pig hippocampal membranes. *Eur. J. Pharmacol.* **137**:293–294 (1987).
28. Martin, K. F., and R. Mason. Ipsapirone is a partial agonist at 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors in the rat hippocampus: electrophysiological evidence. *Eur. J. Pharmacol.* **141**:479–483 (1987).
29. Andrade, R., and R. A. Nicoll. Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **336**:5–10 (1987).
30. Innis, R. B., E. J. Nestler, and G. K. Aghajanian. Evidence for G protein mediation of serotonin- and $GABA_B$-induced hyperpolarization of rat dorsal raphe neurons. *Brain Res.* **459**:27–36 (1988).
31. Bobker, D. H., and J. T. Williams. Ion conductances affected by 5-HT receptor subtypes in mammalian neurons. *Trends Neurosci.* **13**:169–173 (1990).
32. Tricklebank, M. D., C. Forler, and J. R. Fozard. The involvement of subtypes of the 5-HT receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-*n*-propylamino)tetralin in the rat. *Eur. J. Pharmacol.* **106**:271–282 (1985).
33. Smith, L. M., and S. J. Peroutka. Differential effects of 5-hydroxytryptamine$_{1A}$ selective drugs on the 5-HT behavioral syndrome. *Pharmacol. Biochem. Behav.* **24**:1513–1519 (1986).
34. Kucharik, R. F., S. M. White, T. H. Andree, and J. A. Moyer. 5-HT syndrome: differential effects of 5-HT_{1A} selective compounds. *Soc. Neurosci. Abstr.* **14**:555 (1988).
35. Battaglia, G., A. B. Norman, E. J. Hess, and I. Creese. Functional recovery of D1 dopamine receptor-mediated stimulation of rat striatal adenylate cyclase activity following irreversible receptor modification by *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ): evidence for spare receptors. *Neurosci. Lett.* **69**:290–295 (1986).
36. Meller, E., M. Goldstein, A. J. Friedhoff, and J. W. Schweitzer. *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ): a new tool to probe CNS receptor function, in *Central D$_1$ Dopamine Receptors* (M. Goldstein, K. Fuxe, and I. Tabachnick, eds.). Plenum Press, New York, 121–136 (1988).
37. Kenakin, T. P. Receptor reserve as a tissue misnomer. *Trends Pharmacol. Sci.* **7**:93 (1986).
38. Innis, R. B., and G. K. Aghajanian. Pertussis toxin blocks 5-HT_{1A} and $GABA_B$ receptor-mediated inhibition of serotonergic neurons. *Eur. J. Pharmacol.* **143**:195–204 (1987).
39. Clarke, W. P., M. De Vivo, S. G. Beck, S. Maayani, and J. Goldfarb. Serotonin decreases population spike amplitude in hippocampal cells through a pertussis toxin substrate. *Brain Res.* **410**:357–361 (1987).
40. Bohmaker, K., F. Bordi, and E. Meller. The effects of pertussis toxin on dopamine D_2 and serotonin 5-HT_{1A} autoreceptor-mediated inhibition of neurotransmitter synthesis: relationship to receptor reserve. *Neuropharmacology*, in press.

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