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Receptor Reserve for 5-Hydroxytryptamine$_{1A}$-Mediated Inhibition of Serotonin Synthesis: Possible Relationship to Anxiolytic Properties of 5-Hydroxytryptamine$_{1A}$ Agonists

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SUMMARY

The irreversible receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was used to determine the relationship between receptor occupancy and response at central 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) serotonin receptors mediating the inhibition of serotonin synthesis in rat cortex and hippocampus. Rats were treated with vehicle or EEDQ (2 or 6 mg/kg) and 24 hr later dose-response curves were constructed for inhibition of 5-hydroxytrytophan (5-HTP) accumulation (after decarboxylase inhibition with NSD-1015) by the selective 5-HT$_{1A}$ agonists 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (0.01–3 mg/kg), buspirone (0.1–7.5 mg/kg), and ipsapirone (0.1–6.25 mg/kg) and the 5-HT$_{1A}$ agonist/antagonist BMY 7378 (0.015–5 mg/kg). In vehicle-pretreated rats, a similar maximal inhibition of 5-HT synthesis (range, 52–59%) was observed in both brain areas with 8-OH-DPAT, buspirone, and ipsapirone. These three agonists were also more potent in reducing 5-HTP accumulation in the cortex than in the hippocampus (ED_{50}, 8-OH-DPAT, 14 and 30 μg/kg; buspirone, 0.42 and 0.63 mg/kg; ipsapirone, 0.44 and 1.26 mg/kg, respectively). In the cortex, EEDQ treatment shifted the dose-response curves for 8-OH-DPAT, buspirone, and ipsapirone 8.6-, 2.0-, and 2.8-fold to the right, respectively. Corresponding rightward shifts in the hippocampus were smaller, 6.0-, 1.6-, and 2.1-fold, respectively. The EEDQ-induced shifts in the dose-response curves were accompanied by reductions in maximal response. In contrast, whereas the maximal inhibition of cortical 5-HTP accumulation by BMY 7378 (55%) was similar to that obtained with the agonists, maximal response in the hippocampus was much smaller (32%). Furthermore, in both brain regions EEDQ reduced the maximal response to BMY 7378 without shifting the dose-response curves. Analysis of the data by the double-reciprocal method of Furchgott, followed by calculation of fractional receptor occupancy for each dose of agonist, revealed a nonlinear relationship between receptor occupancy and response for 8-OH-DPAT, buspirone, and ipsapirone in both brain regions, demonstrating the presence of a large receptor reserve. For BMY 7378, in contrast, linear relationships were obtained. Because 5-HT$_{1A}$ receptor-mediated regulation of 5-HT synthesis appears to be mediated by somatodendritic autoreceptors on 5-HT neurons in the midbrain raphé nuclei, the results suggest that these autoreceptors possess a large receptor reserve for agonists. The relevance of these findings for the mechanism of action of nonbenzodiazepine anxiolytics is discussed.

Receptor binding studies have been instrumental in classifying 5-HT receptors into at least three broad categories, 5-HT$_1$, 5-HT$_2$, and 5-HT$_3$ (1). Further subclassification of 5-HT$_1$ receptors as 5-HT$_{1A}$ (2, 3), 5-HT$_{1B}$ (4), 5-HT$_{1C}$ (5), and 5-HT$_{1D}$ (6) has been accomplished utilizing subtype-selective agonists and antagonists, and functional correlates of these 5-HT$_1$ receptor subtypes have been described (see Ref. 1). Somatodendritic 5-HT$_{1A}$ autoreceptors on serotonin neurons in the midbrain raphé nuclei mediate inhibition of their spontaneous activity (7–10). New nonbenzodiazepine anxiolytics such as buspirone, ipsapirone, and gepirone (11) are selective agonists at 5-HT$_{1A}$ receptors (3, 11, 12) and potently and completely inhibit 5-HT neuronal firing (7–10), leading to the suggestion that the latter functional effect is responsible for their anxiolytic properties (11, 13).

Whereas 5-HT$_{1A}$ receptors in the raphé nuclei are localized to serotonergic neurons (14), in terminal fields (e.g., in hippocampus and cortex) they appear to occur only on postsynaptic cells (15). Interestingly, the potency and efficacy of the nonbenzodiazepine anxiolytics differ markedly in pre- and postsynaptic paradigms of 5-HT$_{1A}$-mediated receptor function. Elec-

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; BMY 7378, 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4.5]decane-7,9-dione dihydrochloride; SCH 23390, 8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzapin-7-ol; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; NSD-1015, *m*-hydroxybenzylhydrazine; LSD, lysergic acid diethylamide.

trophysiological studies have shown them to be potent agonists in the raphé (9, 10, 16, 17), similar to the effects of 5-HT and the prototypical 5-HT_{1A} agonist 8-OH-DPAT, but partial agonists at the postsynaptic 5-HT_{1A} receptor in the rat hippocampus (16, 18, 19). These agents also display partial agonism for 5-HT_{1A}-mediated inhibition of forskolin-stimulated adenylate cyclase in rat and guinea pig hippocampal membranes, whereas 8-OH-DPAT appears to be a full agonist (20). A buspirone analog, BMY 7378, displays very low intrinsic activity and behaves as an antagonist in this system (21). Behaviorally, 8-OH-DPAT elicits the 5-HT syndrome (22–24), which reflects postsynaptic 5-HT_{1A} receptor activation (24), but only at doses which are markedly higher than those required to suppress neuronal firing in the raphé nuclei (10, 17). On the other hand, the nonbenzodiazepine anxiolytics only weakly induce the 5-HT syndrome and, in fact, partially antagonize the effects of 8-OH-DPAT and other more efficacious agonists (23, 25, 26).

These observations afford a striking parallel to those seen for DA agonists in the nigrostriatal system. DA agonists are generally more potent at pre- than postsynaptic D2 receptors, and weak partial DA agonists display almost total selectivity for presynaptic receptors (see Ref. 27). We have shown that a large receptor reserve for agonists exists at terminal DA autoreceptors (27, 28), whereas postsynaptic D2 receptors have no receptor reserve (29). We have suggested that this differential receptor reserve is the molecular basis for the autoreceptor selectivity of DA agonists (27, 28), because weak partial agonists display substantial or even full intrinsic activity in the presence of a large receptor reserve, relative to its absence (30).

In view of the similar differential effects of the respective agonists at pre- and postsynaptic D2 and 5-HT_{1A} receptors, it seemed likely that a similar difference in the extent of receptor reserve at pre- versus postsynaptic 5-HT_{1A} receptors might account for the observed effects of the nonbenzodiazepine anxiolytics. In addition to the functional effects described above, 5-HT_{1A} agonists also reduce 5-HT synthesis in cortex and hippocampus after systemic treatment (22, 31–33). This effect appears to be mediated by their action at somatodendritic autoreceptors because: 1) terminal autoreceptors are not of the 5-HT_{1A} type (see above); 2) serotonin synthesis, in contrast to DA synthesis, appears primarily to be under control of impulse flow (34); 3) axotomy of ascending serotonergic pathways abolishes the inhibitory effects of 8-OH-DPAT (31, 32) and ipsapirone (32) on serotonin turnover; and 4) direct intra-raphé injection of 8-OH-DPAT (31, 32) and ipsapirone (32) mimics the effects of systemic treatment. We, therefore, examined the relationship between receptor occupancy and response for this effect by constructing dose-response curves for inhibition of 5-HT synthesis in rat cortex and hippocampus by several 5-HT_{1A} agonists before and after partial irreversible 5-HT receptor inactivation with EEDQ (27, 35). The results demonstrate that, indeed, a large receptor reserve for full 5-HT_{1A} agonists exists at the somatodendritic 5-HT_{1A} autoreceptor.

Experimental Procedures

Materials. Drugs and chemicals were obtained from the following sources. EEDQ, NSD-1015, 5-HTP, 5-HT, and 5-HIAA were obtained from Sigma Chemical Co. (St. Louis, MO). 8-OH-DPAT HBr was purchased from Research Biochemicals (Natick, MA). Buspirone HCl was a gift from Mead Johnson and Co. (Evansville, IN), now available from Bristol-Myers Co. (Wallingford, CT), which also supplied BMY 7378. Ipsapirone was a generous gift of Miles, Inc. (West Haven, CT). Racemic sulpiride HCl and SCH 23390 were obtained from Ravizza (Milan, Italy) and Schering-Plough Corp. (Bloomfield, NJ), respectively. All other reagents were of the highest chemical purity commercially available.

Drug treatments. Male Sprague-Dawley rats (180–280 g) were maintained on a 12-hr light/dark cycle and housed six/cage with food and water *ad libitum*. Groups of animals were treated with EEDQ (2 or 6 mg/kg, subcutaneously) or vehicle. Twenty-four hours later, groups of rats received various doses of 8-OH-DPAT (0.01–3 mg/kg, subcutaneously), buspirone (0.1–7.5 mg/kg, subcutaneously), ipsapirone (0.1–6.25 mg/kg, subcutaneously), BMY 7378 (0.015–5 mg/kg, subcutaneously), or vehicle 30 min before treatment with the decarboxylase inhibitor NSD-1015 (100 mg/kg, intraperitoneally). Animals were sacrificed by decapitation 30 min after NSD-1015 treatment and the entire hippocampus and both hemispheres of the cerebral cortex were dissected on ice and stored at −80° for up to 2 weeks before assay. In receptor protection experiments, groups of rats were treated, 24 hr before drug challenge, with vehicle alone, EEDQ (6 mg/kg) alone, BMY 7378 (10 mg/kg) 30 min before EEDQ, or a combination of sulpiride (150 mg/kg) and SCH 23390 (0.2 mg/kg) 150 and 30 min, respectively, before EEDQ.

5-HTP Assay. Tissues were homogenized, in 0.1 N perchloric acid containing 0.001% ascorbic acid, with a Brinkmann Polytron at setting 5 for 25 sec. Tissue concentrations were 100 mg/ml for cortices and 50 mg/ml for hippocampi. An aliquot of homogenate was transferred to a 1.5-ml Eppendorf tube and centrifuged for 12 min in an Eppendorf microfuge. After centrifugation, 20-μl aliquots of the supernatant were injected onto an ESA high performance liquid chromatography system consisting of a model 5100A Coulometric electrochemical detector equipped with a model 5011 dual electrode high sensitivity analytical cell. The working electrode potentials for the coulometric-amperometric analytical cell were +0.05 and −0.40 V respectively. The high performance liquid chromatography system was fitted with an ESA catecholamine HR-80 column and analyses were run at ambient temperature. The mobile phase was ESA CAT-A-PHASE (pH 2.56) containing 5% methanol and 0.003% sodium octyl sulfate, at a flow rate of 1.5 ml/min.

Data analysis. Dose-response curves for drug-induced inhibition of 5-HTP accumulation in cortex and hippocampus after vehicle or EEDQ pretreatment were simultaneously analyzed for best fit using the ALL-FIT computer program of De Lean *et al.* (36), as described previously in detail (27–29). Briefly summarized, the program provided statistical tests of the goodness of fit after the curves were constrained to share one or more parameters (response at zero dose, slope factor, 50% of maximally effective dose or ED_{50}, and response at "infinite" dose). In practice, the response at zero dose for all curves was set to zero. Curves were first analyzed without constraints and then by successively constraining them to share a common slope factor, ED_{50}, or maximal response. That analysis which permitted one or more of the parameters to be shared without a significant increase in the residual variance (27, 36) was taken as the best fit.

Pseudo-dissociation constants (K_A values, in units of dose; see Ref. 27) for agonist-induced reductions in 5-HTP accumulation were obtained by the method of Furchgott and Bursztyn (37), 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 specific 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, (i.e., not inactivated). Pseudo-K_A values were obtained by plotting the reciprocals of the equieffective doses of agonist after inactivation, $1/[A']$, against the reciprocals of the doses before inactivation, $1/[A]$, for each pair of dose-response curves in each tissue. The equieffective doses were determined at five levels of re-

sponse (corresponding to 30, 40, 50, 60, and 70% of the maximum effect after EEDQ treatment) (27, 37) from the ALLFIT-derived best-fit dose-response curves. Each resulting straight line had a slope of $1/q$ and pseudo-K_A equal to (slope − 1)/y-intercept.

The pseudo-K_A values in units of dose were used to calculate fractional receptor occupancy (f) at a particular dose [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 or total concentration of active receptors. Fractional receptor occupancy at a particular dose was then plotted against fractional response at that dose (obtained from the control best fit dose response curve).

Results

Effects of EEDQ on basal 5-HTP accumulation. EEDQ treatment (6 mg/kg) did not significantly affect the NSD-1015-induced accumulation of 5-HTP in rat hippocampus [ng/g wet weight tissue ± SE: vehicle, 142.8 ± 8.1 (n = 10); EEDQ, 144.7 ± 7.4 (n = 11)]. In contrast, a significant effect was observed in the cortex [vehicle, 84.2 ± 3.7 (n = 10); EEDQ, 127.4 ± 6.2 (n = 11); $p < 0.001$]. Because EEDQ irreversibly and nonselectively blocks 5-HT receptors in the brain (35), this effect may reflect a negative feedback control of cortical but not hippocampal 5-HT synthesis, although the existence of such a feedback mechanism is still a matter of some controversy (31, 38).

8-OH-DPAT-induced inhibition of 5-HTP accumulation after partial irreversible receptor inactivation. In the cortex, EEDQ treatment produced a dramatic 8.6-fold shift to the right in the dose-response curve for inhibition of 5-HTP accumulation by 8-OH-DPAT [ED_{50} (mg/kg): vehicle, 0.014; EEDQ, 0.121], as well as a relatively small depression in the maximal response (vehicle, 55.2%; EEDQ, 40.0%) (Fig. 1). A similar but somewhat smaller 6.1-fold shift was observed in the hippocampus [ED_{50} (mg/kg): vehicle, 0.030; EEDQ, 0.180], along with a slightly larger relative reduction in the maximal response (vehicle, 52.4%; EEDQ, 34.0%). The large rightward shifts in the dose-response curves coupled with only small depressions in maximal response immediately suggest the presence of a substantial receptor reserve (27, 30, 37). Furthermore, the approximately 2-fold greater potency of 8-OH-DPAT in reducing cortical 5-HTP accumulation suggests that there is an even larger receptor reserve at those 5-HT$_{1A}$ receptors regulating 5-HT synthesis in the cortex than in the hippocampus; the larger rightward shift, after EEDQ treatment, in the dose-response curve for 8-OH-DPAT in the cortex is also consistent with this suggestion. These results imply that different subpopulations of 5-HT$_{1A}$ receptors may mediate the effects of 8-OH-DPAT in these regions (see Discussion).

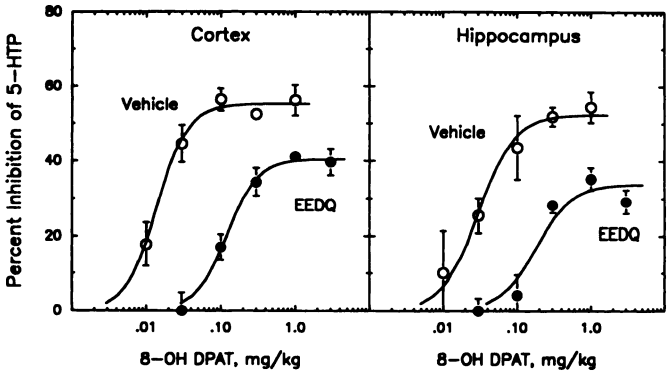

Fig. 1. Dose-response curves for inhibition by 8-OH-DPAT of 5-HTP accumulation in cortex and hippocampus of vehicle- and EEDQ (6 mg/kg)-pretreated rats. All animals were treated with NSD-1015 (100 mg/kg) 30 min before sacrifice. The best-fit curves (——) were obtained by simultaneous ALLFIT analysis of each pair of curves. Each *point* is the mean ± standard error of four to seven animals.

Effects of buspirone on 5-HTP accumulation after partial irreversible receptor inactivation. In contrast to its effects on 8-OH-DPAT, EEDQ treatment elicited a much smaller (2.0-fold) rightward shift in the dose-response curve for buspirone-induced inhibition of 5-HTP accumulation in the cortex [ED_{50} (mg/kg): vehicle, 0.42; EEDQ, 0.82] (Fig. 2). The maximal response was also reduced (vehicle, 53.6%; EEDQ, 44.4%). As with 8-OH-DPAT, EEDQ produced a smaller rightward shift in the hippocampus (1.6-fold) [ED_{50} (mg/kg): vehicle, 0.63; EEDQ, 1.01]; the maximal response was also depressed (vehicle, 55.1%; EEDQ, 42.9%). Buspirone was slightly more potent in the cortex than in the hippocampus (1.5-fold), as was seen with 8-OH-DPAT.

Ipsapirone-induced inhibition of 5-HTP accumulation. The magnitude of EEDQ treatment effects on ipsapirone-induced inhibition of 5-HTP accumulation (Fig. 3) was qualitatively similar to that seen with buspirone. In the cortex, a moderate (2.8-fold) rightward shift in the ED_{50} was observed (vehicle, 0.44; EEDQ, 1.23 mg/kg) as well as a reduced maximal response (vehicle, 58.6%; EEDQ, 43.5%). As was seen with both

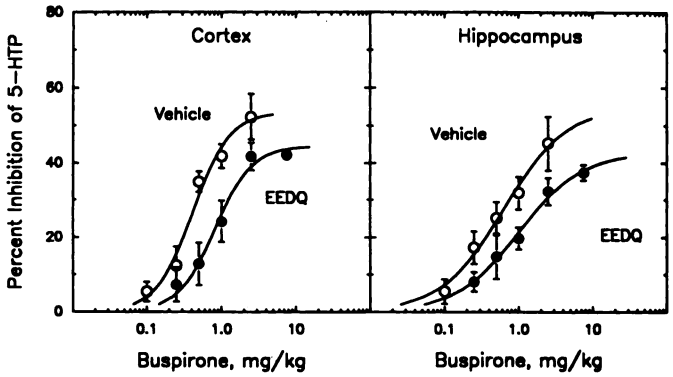

Fig. 2. Dose-response curves for buspirone-induced inhibition of 5-HTP accumulation in rat cortex and hippocampus after vehicle and EEDQ (6 mg/kg) treatment. Each *point* is the mean ± standard error of five to seven animals.

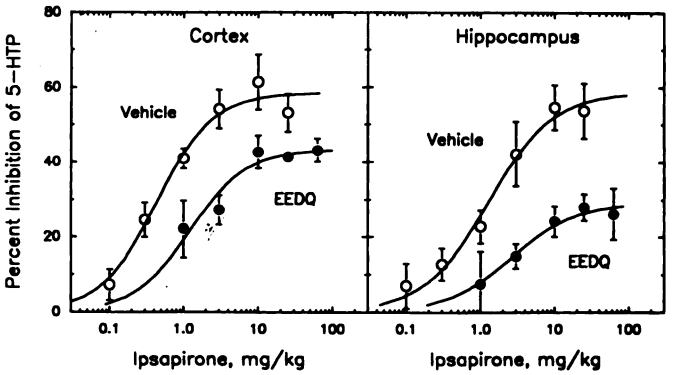

Fig. 3. Dose-response curves for ipsapirone-induced inhibition of 5-HTP accumulation in rat cortex and hippocampus after vehicle and EEDQ (6 mg/kg) treatment. Each *point* is the mean ± standard error of four to six rats.

8-OH-DPAT and buspirone, EEDQ produced a smaller (2.1-fold) rightward shift (ED_{50}: vehicle, 1.26; EEDQ, 2.64 mg/kg) and greater loss of maximal response (vehicle, 58.8%; EEDQ, 29.3%) in the hippocampus; like the other agonists, ipsapirone was more potent (2.9-fold) in the cortex than in the hippocampus.

It should be noted that the control maximal inhibition of 5-HTP accumulation obtained was of similar magnitude (range, 52–59%), irrespective of agonist or brain region. A comparable degree of maximal response independent of brain area has been reported previously (31–33).

BMY 7378-induced inhibition of 5-HTP accumulation. Although BMY 7378 has low intrinsic activity at postsynaptic 5-HT_{1A} receptors and antagonizes 5-HT_{1A} agonist-mediated inhibition of adenylate cyclase in rat and guinea pig hippocampal membranes (21), it nevertheless suppresses 5-HT neuronal activity in the dorsal raphé (39). Not surprisingly, therefore, BMY 7378 also inhibited 5-HTP accumulation in the cortex (Fig. 4), to an extent (55%) similar to that observed with the other drugs. However, the maximal response in the hippocampus was much smaller (32.4%; Fig. 4, *right*). In further contrast to the other drugs, EEDQ treatment (2 mg/kg) did not shift the dose-response curves for BMY 7378 in either cortex or hippocampus; rather, only the maximal responses were reduced (to 34.0 and 20.4%, respectively). Finally, again unlike the other agents tested, the ED_{50} of BMY 7378 was nearly identical in both brain regions (Fig. 4).

Relationship between receptor occupancy and response. For each drug, in each brain region, each pair of dose-response curves were analyzed by the double-reciprocal method of Furchgott and Bursztyn (37), as described previously (27–29), to yield the pseudo-K_A and q values shown in Table 1. The pseudo-K_A values were then used to calculate fractional receptor occupancy, which is shown plotted against response in Fig. 5. In the cortex, there is a very steep hyperbolic relationship for 8-OH-DPAT, with maximal response (95–100%) requiring approximately 20% receptor occupancy, i.e., there is an 80% receptor reserve for 8-OH-DPAT-induced inhibition of 5-HTP accumulation in cortex. As suggested by the lower potency of 8-OH-DPAT in the hippocampus, the receptor reserve for this agonist to elicit response in this region is smaller, about 60–70%. It is also clear that both buspirone and ipsapirone are partial agonists relative to 8-OH-DPAT in both regions, because the relationship between receptor occupancy and response is progressively flattened for these agonists. For BMY 7378, the relationship is best described by a linear regression of the experimental points, i.e., there is no receptor reserve for this drug. The percentage of receptor occupancy required to elicit 50% of the maximal response for each agonist in each tissue is shown in Table 1; the efficacies of the drugs relative to 8-OH-DPAT are similar in both tissues, as expected if the response to each drug results from interaction with the same receptor (27, 30).

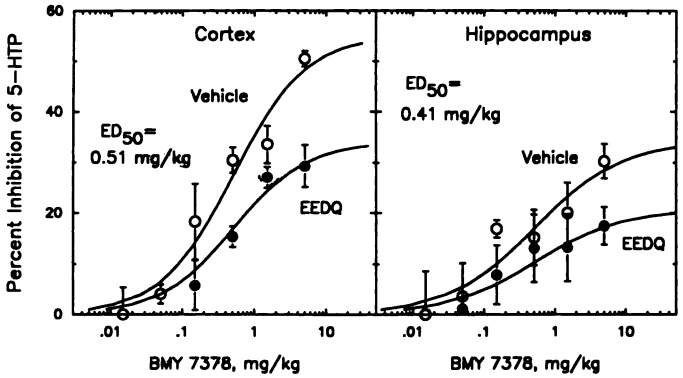

Fig. 4. Dose-response curves for BMY 7378-induced inhibition of 5-HTP accumulation in rat cortex and hippocampus after vehicle and EEDQ (2 mg/kg) treatment. A lower dose of EEDQ was used in this experiment because it was anticipated that the usual dose (6 mg/kg) would reduce response in the hippocampus to levels too low to allow analysis by the Furchgott method. Each *point* is the mean ± standard error of four or five rats.

Effects of protecting 5-HT_{1A} or D1 plus D2 dopamine receptors from inactivation by EEDQ. EEDQ irreversibly inactivates D1 and D2 receptors as well as 5-HT receptors (40). Buspirone and ipsapirone, but not 8-OH-DPAT, also have moderate affinity for DA receptors and affect DA synthesis (32). It is possible, therefore, that the effects of these drugs on 5-HTP accumulation in EEDQ-treated animals could be modulated by the concomitant loss of D1 and/or D2 receptors. We, therefore, compared the extent of inhibition of 5-HTP accumulation elicited by 8-OH-DPAT and buspirone in animals pretreated with either BMY 7378 (10 mg/kg) or sulpiride (150 mg/kg) plus SCH 23390 (0.2 mg/kg) (40) before EEDQ to protect 5-HT_{1A} or D2 plus D1 receptors, respectively, from inactivation. Fig. 6 shows that BMY 7378 pretreatment prevented the EEDQ-induced loss of response to either 8-OH-DPAT or buspirone, whereas protecting D1 and D2 receptors from inactivation resulted in response that was indistinguishable from that elicited in animals treated with EEDQ alone. These results further support the suggestion that the effects of these agents on 5-HTP accumulation derive from specific interaction with 5-HT_{1A} receptors. Electrophysiological studies have similarly concluded that the DAergic properties of buspirone are not involved in its suppressive effect on 5-HT neuronal activity in the dorsal raphé (17).

Discussion

The findings presented here demonstrate that there is a large receptor reserve for 5-HT_{1A}-mediated inhibition of 5-HT synthesis in the cortex and hippocampus. This explains why buspirone, ipsapirone, and BMY 7378, which clearly are partial agonists relative to 8-OH-DPAT (Table 1), nevertheless display full intrinsic activity in control animals (except for BMY 7378 in hippocampus). Partial agonists can elicit maximal apparent response in the presence of a receptor reserve (27, 30, 37). In this respect, these results are comparable to those obtained for partial DA agonists at the DA autoreceptor (27, 28).

While a determination of the relationship between receptor occupancy and response at postsynaptic 5-HT_{1A} receptors in various terminal areas of ascending serotonergic pathways is currently underway, it is likely, in analogy with the situation for D2 DA receptors (27–29), that a differential receptor reserve at somatodendritic and postsynaptic 5-HT_{1A} receptors may underlie the pharmacological differences observed at these sites with 5-HT_{1A} agonists (see Introduction). If, as postulated, postsynaptic 5-HT_{1A} receptors in terminal areas have little receptor reserve for agonists, then partial agonists such as buspirone and ipsapirone may be exerting their anxiolytic effects via a preferential inhibition of impulse flow in serotonergic neurons in the raphé nuclei, with considerably less efficacy at postsyn-

TABLE 1

Receptor parameters for 5-HT$_{1A}$ agonists and antagonists in cortex and hippocampus

Pseudo-K_A and q values were obtained by plotting the reciprocals of the equieffective doses of the agonists required to elicit identical levels of inhibition of 5-HTP accumulation before and after treatment with EEDQ. Doses were obtained at five levels of effect (30–70% of the maximal effect in EEDQ-treated rats) from the best-fit ALLFIT-derived curves (Figs. 1–4). Percentages of receptor occupancy required to elicit 50% of the maximal effect were obtained from the data in Fig. 5. Relative efficacy values for buspirone, ipsapirone, and BMY 7378 were obtained from the ratios of the percentage of receptor occupancy required for 50% response for 8-OH-DPAT over that required for each drug in each tissue. Although the fraction of receptors (q) left intact after the same EEDQ treatment was different in the 8-OH-DPAT, buspirone, and ipsapirone experiments, this does not affect the results obtained. Similar different degrees of receptor inactivation by the same dose of EEDQ have been noted previously (49, 50).

Drug	Brain region	Pseudo-K_A	q	Occupancy at 50% of maximal response	Relative efficacy
		mg/kg		%	
8-OH-DPAT	Cortex	0.41	0.11	3.3	1.0
	Hippocampus	0.43	0.15	6.5	1.0
Buspirone	Cortex	2.43	0.49	14.6	0.23
	Hippocampus	2.34	0.49	21.2	0.31
Ipsapirone	Cortex	3.45	0.28	11.2	0.29
	Hippocampus	4.02	0.24	23.9	0.27
BMY 7378	Cortex	0.59	0.55	46.4	0.07
	Hippocampus	0.56	0.47	42.3	0.15

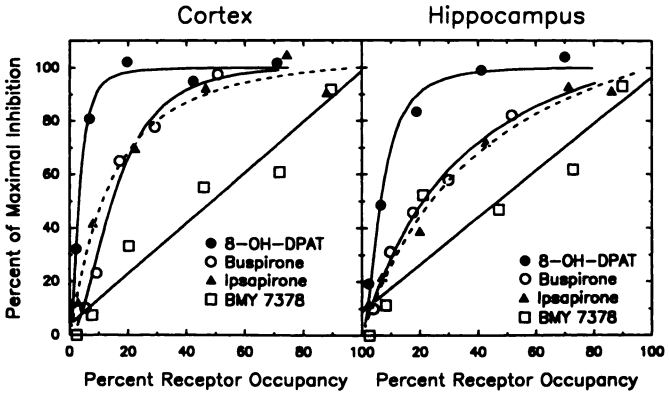

Fig. 5. Maximal inhibition of 5-HT synthesis in cortex and hippocampus by 8-OH-DPAT, buspirone, ipsapirone, and BMY 7378 as a function of receptor occupancy. Fractional receptor occupancy (f) for the agonists was calculated from the law of mass action ($f = [A]/K_A + [A]$), using the pseudo-K_A values shown in Table 1 and the corresponding ALLFIT-derived vehicle dose-response curves (Figs. 1–3), as described in Experimental Procedures. The BMY 7378 data points were subjected to linear regression ($r = 0.97$ in cortex, 0.92 in hippocampus).

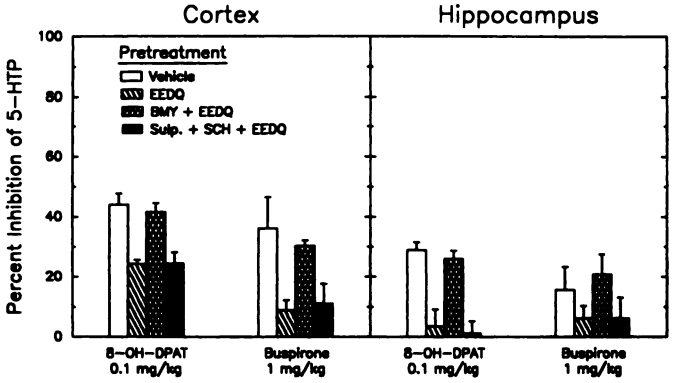

Fig. 6. Effects of protecting 5-HT$_{1A}$ or D1 plus D2 receptors on the response to 8-OH-DPAT and buspirone. For each agonist, four groups of rats were treated with vehicle alone, EEDQ alone, BMY 7378 and EEDQ, or sulpiride plus SCH 23390 and EEDQ according to the dose and time schedule described in Experimental Procedures. Twenty-four hours later each group was subdivided; one subgroup received NSD-1015 alone and the other received agonist plus NSD-1015. Results are expressed as percentage of inhibition of 5-HTP accumulation relative to NSD-1015 alone. n = 6–9 animals/group for 8-OH-DPAT and 4 or 5 for buspirone. For 8-OH-DPAT in cortex and hippocampus, and buspirone in cortex, the groups treated with EEDQ alone or sulpiride plus SCH 23390 and EEDQ were significantly ($p < 0.05$) different from vehicle, whereas the groups treated with BMY 7378 and EEDQ were not (Dunnett's test). The data for buspirone in the hippocampus were not significantly different, due to the low level of response and large variance.

aptic sites. In this regard, it is interesting to note that 8-OH-DPAT, which has substantially greater efficacy than buspirone and ipsapirone (Table 1), has generally been reported to lack anxiolytic activity (Refs. 11 and 41; but see also Ref. 42). This may reflect significant postsynaptic agonist efficacy of 8-OH-DPAT, which presumably would counteract its somatodendritic anxiolytic effects. Supporting this hypothesis is the observation that direct injection of 8-OH-DPAT into the median raphé elicited anxiolytic behavioral effects, whereas systemic treatment (which we hypothesize would activate both pre- and postsynaptic receptors) did not (41). [Interestingly, systemic 8-OH-DPAT did display anxiolytic effects when tested under conditions when 5-HT release and turnover are known to be elevated; this has been interpreted as reflecting conditions that favor the autoreceptor-mediated inhibitory effects of 8-OH-DPAT (41)]. Additional support for this hypothesis is provided by the recent finding that BMY 7378 displays behavioral effects in animals predictive of anxiolytic properties (43), yet it is a very weak partial agonist with very low intrinsic activity at postsynaptic 5-HT$_{1A}$ receptors (21). However, it shares with the other agonists the ability to suppress both 5-HT neuronal activity in the raphé nuclei (39) and transmitter synthesis in terminal projection areas (Fig. 4). Moreover, these data support the suggestion (see Introduction) that the drug-induced inhibition of 5-HT synthesis produced by these agents is mediated by somatodendritic 5-HT$_{1A}$ autoreceptors.

Potential differences in receptor reserve at other 5-HT receptor subtypes, not only between pre- and postsynaptic sites but also among postsynaptic receptors in different brain regions, may have some bearing on puzzling findings of long standing regarding the effects of 5-HT agonists and antagonists in electrophysiological studies (44). LSD, for example, completely inhibits the firing of dorsal raphé neurons but usually accelerates the firing of postsynaptic cells (e.g., in the ventral lateral geniculate), in contrast to 5-HT itself, which also inhibits postsynaptic neuronal firing (44). The known partial agonist properties of LSD, coupled with potentially large differences in

receptor reserve at these sites, may help explain this finding, although its complex properties may as readily derive from its similar affinity for multiple receptor subtypes (45). Furthermore, most 5-HT antagonists mimic the depressant response to 5-HT in the raphé without blocking the latter's effects; their effects on postsynaptic responses are very complex and differ substantially from one region to another (44). Although these complex effects are likely due in part to the presence of different 5-HT receptor subtypes in these areas and to differences in affinity of the various drugs at these subtypes, it is also possible that differences in receptor reserve and drug efficacy play a significant role. In particular, some of these antagonists may have very weak partial agonist efficacy; they might, therefore, elicit a substantial agonist response at sites exhibiting a large receptor reserve but display antagonist properties at sites lacking a receptor reserve (27). Because a variety of new agents with varying degrees of selectivity for these 5-HT subtypes are now available, and because EEDQ is probably an effective irreversible ligand for all 5-HT receptors (35, 40), a fresh examination of these issues utilizing these tools is likely to provide new and important insights into serotonergic function.

The greater potency of 8-OH-DPAT, buspirone, and ipsapirone for inhibiting cortical than hippocampal 5-HT synthesis and the larger receptor reserve for the cortical response (i.e., occupancy of approximately half as many receptors is necessary to elicit half-maximal response in cortex than in hippocampus; see Table 1) deserve some comment. These results suggest, as mentioned earlier (see Results), that different autoreceptor populations may subserve the effects of the agonists in these regions. Consistent with such an interpretation, the hippocampus receives its serotonergic innervation almost exclusively from the median raphé, whereas the cortex is innervated by axons arising from both the dorsal and median nuclei (46). This suggests that dorsal raphé 5-HT autoreceptors have an even larger receptor reserve than is apparent in this study, because the cortical response is probably contaminated by the effects of the agonists at the less sensitive autoreceptors in the median raphé, which have a smaller receptor reserve. Indeed, a recent electrophysiological study reported a 30-fold greater potency for 8-OH-DPAT in suppressing the firing of dorsal than median raphé 5-HT neurons (10). Experiments are in progress to test this hypothesis by examining the effects of these and other agonists on 5-HT synthesis in areas receiving a predominant innervation from the dorsal raphé [e.g., the corpus striatum (46)].

The serotonergic projections from these midbrain nuclei are also distinguishable neuroanatomically; very fine 5-HT axons with minute varicosities arise from the dorsal raphé, whereas those from the median raphé are beaded in appearance because of the presence of large varicosities (47). These two axonal types are also pharmacologically distinguishable, because the fine axons degenerate after treatment with the neurotoxic amphetamine derivatives methylenedioxyamphetamine and *p*-chloroamphetamine while the beaded ones are spared (48). The present results provide additional evidence for a functional differentiation of these serotonergic projections.

In conclusion, we have found a large receptor reserve for 5-HT_{1A} agonist-induced inhibition of 5-HT synthesis in rat brain; this response appears to be mediated by somatodendritic autoreceptors on 5-HT neurons in the raphé nuclei. This finding is rich in implications for our understanding of the mechanisms involved in the anxiolytic properties of nonbenzodiazepine agents such as buspirone, ipsapirone, and gepirone. Analogous studies examining the relationship between receptor occupancy and response at postsynaptic 5-HT_{1A} receptors will provide a critical test of the hypothesis that a differential receptor reserve at somatodendritic versus postsynaptic sites underlies the anxiolytic properties of these agents. The method described here also provides a simple procedure that may turn out to be useful for identifying potential anxiolytic drugs and determining their relative efficacies at 5-HT_{1A} receptors.

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