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European Journal of Pharmacology 329 (1997) 99–106

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[^{3}H]8-OH-DPAT labels a 5-HT site coupled to inhibition of phosphoinositide hydrolysis in the dorsal raphe

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Received 24 February 1997; revised 10 April 1997; accepted 15 April 1997

Abstract

The present study was undertaken to compare the properties of the [^{3}H]8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) binding site in the dorsal raphe nucleus with the hippocampal 5-HT$_{1A}$ receptor. In both tissues inclusion of 1 mM Mg^{2+} enhanced specific [^{3}H]8-OH-DPAT binding, while 1 mM GTP decreased radioligand binding. [^{3}H]8-OH-DPAT appears to bind to a single population of binding sites in both the hippocampus and the dorsal raphe nucleus, although the K_d for the radioligand at the dorsal raphe site was five times that observed at the hippocampal 5-HT$_{1A}$ receptor. Similarly, although 5-HT and selective 5-HT$_{1A}$ receptor ligands displayed high affinity for the [^{3}H]8-OH-DPAT binding site in the dorsal raphe nucleus, the affinity at the dorsal raphe site was less than that observed at the hippocampal 5-HT$_{1A}$ receptor. 8-OH-DPAT inhibited forskolin-stimulated adenylyl cyclase activity in the hippocampus, but did not alter enzyme activity in the dorsal raphe nucleus. Conversely, 8-OH-DPAT inhibited the accumulation of [^{3}H]inositol phosphates in the dorsal raphe nucleus, but not in the hippocampus. An inhibition of phosphoinositide hydrolysis in the dorsal raphe nucleus also was found with the putative 5-HT$_{1A}$ receptor selective ligands, flesinoxan and gepirone. However, addition of another putative 5HT$_{1A}$ receptor selective ligand, buspirone, did not alter the generation of [^{3}H]inositol phosphates, but blocked the inhibitory effect of 8-OH-DPAT on phosphoinositide hydrolysis. These studies demonstrate that the 8-OH-DPAT binding site in the dorsal raphe nucleus displays a binding profile which is similar to the hippocampal 5-HT$_{1A}$ receptor, but unlike this 5-HT$_{1A}$ receptor the binding site in the dorsal raphe nucleus is negatively coupled to phosphoinositide turnover. © 1997 Elsevier Science B.V.

Keywords: 5-HT receptor; Adenylyl cyclase; Phosphoinositide hydrolysis; Raphe nucleus, dorsal; Hippocampus; 5-HT$_{1A}$ receptor

1. Introduction

The 5-hydroxytryptamine (5-HT) receptor can be classified into at least seven major classes labeled 5-HT$_1$ through 5-HT$_7$ with further subdivisions of some of the classes such as the 5-HT$_1$ class into A, B–D, E, F (Zifa and Fillion, 1992). The 5-HT$_{1A}$ receptor belongs to the large family of biogenic amine receptors coupled to G proteins (Peroutka, 1993) and appears to be involved in many of the physiological (e.g., thermoregulation, mood, sleep) and pathophysiological (e.g., anxiety, depression, alcoholism) actions of 5-HT (Zifa and Fillion, 1992). Using radiolabeled 8-hydroxy-2(di-*n*-propylamino)tetralin (8-OH-DPAT), a selective 5-HT$_{1A}$ receptor agonist, autoradiographic studies have identified the hippocampus and dorsal raphe nucleus as having the highest densities of 5-HT$_{1A}$ receptors (Marcinkiewicz et al., 1984; Pazos and Palacios, 1985; Gozlan et al., 1988). The sites labeled by 8-OH-DPAT in the dorsal raphe nucleus are located presynaptically on serotonergic cell bodies and function as autoreceptors (somatodendritic autoreceptors) whereas the hippocampal sites are predominantly postsynaptic (Verge et al., 1985; Weissmann-Nanopoulos et al., 1985). Activation of the 5-HT$_{1A}$ receptors in the dorsal raphe appears to be preferentially involved in the hyperphagic and anxiolytic actions of 5-HT$_{1A}$ receptor agonists (Bendotti and Samanin, 1986; Hutson et al., 1986; Carli and Samanin, 1988), while the hypothermic effect and components of the 5-HT syndrome appear to involve the postsynaptic receptors (Tricklebank et al., 1984, 1985; Hutson et al., 1987). Both the presynaptic receptor in the dorsal raphe nucleus and the postsynaptic hippocampal receptors are sensitive to pertussis toxin indicating both receptors are coupled to G_i or G_o (Andrade et al., 1986; Innis et al., 1988; Blier et al., 1993b). Several studies have shown that the hippocampal

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PII S0014-2999(97)00136-2

5-HT_{1A} receptor is coupled through a G_i protein to an inhibition of adenylyl cyclase activity (De Vivo and Maayani, 1986; Varrault et al., 1991; Chamberlain et al., 1993). Activation of the hippocampal and dorsal raphe 5-HT_{1A} receptors also was reported to cause a hyperpolarization presumably through an increase in K^+ conductance (Aghajanian and Lakoski, 1984; Williams et al., 1988; Beck et al., 1992).

In addition to the difference in subcellular localization, pharmacological differences between the 5-HT_{1A} receptors in the hippocampus and dorsal raphe nucleus also have been reported. Administration of selective 5-HT reuptake inhibitors and 5-HT_{1A} receptor agonists elicits a desensitization of the somatodendritic receptor in the dorsal raphe without any change in the hippocampal 5-HT_{1A} receptor (Chaput et al., 1986; Blier and De Montigny, 1990; Schechter et al., 1990; Godbout et al., 1991). The 5-HT_{1A} receptors in the hippocampus and dorsal raphe nucleus differ in sensitivity to the effects of cholera toxin and acute spiperone administration (Blier et al., 1993a,b). The antagonists, WAY 100635 and pindolol, also displayed different characteristics at the 5-HT_{1A} receptor in the hippocampus and dorsal raphe nucleus (Corradetti et al., 1996; Romero et al., 1996). Furthermore, several compounds function as full agonists at 5-HT_{1A} receptors in the dorsal raphe, but only as partial agonists at 5-HT_{1A} receptors in the hippocampus (Andrade and Nicoll, 1987; Williams et al., 1988; Sprouse and Aghajanian, 1988; Blier and De Montigny, 1990; Godbout et al., 1991). Although some of these pharmacological differences between the hippocampal and dorsal raphe 5-HT_{1A} receptors may be explained by the difference in the amount of receptor reserve (Meller et al., 1990; Yocca et al., 1992), other explanations such as different isoforms of the 5-HT_{1A} receptor (Albert et al., 1990) or differences in signal transduction (Fargin et al., 1989) also may be valid.

The present study was undertaken to compare the [^{3}H]8-OH-DPAT binding properties and signal transduction system in the dorsal raphe nucleus and hippocampus. Our results indicate that the [^{3}H]8-OH-DPAT binding site in the dorsal raphe nucleus shares similar binding properties to the hippocampal 5HT_{1A} receptor. However, unlike the hippocampal 5-HT_{1A} receptor the 8-OH-DPAT binding site in the dorsal raphe nucleus is not coupled to adenylyl cyclase but instead is negatively coupled to phosphoinositide turnover.

2. Materials and methods

2.1. Tissue isolation

Male Fisher 344 rats were killed by decapitation, and their brains quickly removed. For binding studies the dorsal raphe nucleus was isolated as previously described (Johnson et al., 1996). Briefly, the brain was blocked at the level of the striatum and the cerebellum and was frozen on a microtome stage. Two 1 mm thick coronal slices were made through the brainstem between the caudal portion of the inferior colliculi, −9.30 mm from bregma (Paxinos and Watson, 1986), and the medial geniculate nucleus, −6.30 mm from bregma (Paxinos and Watson, 1986). The dorsal raphe nucleus was removed with a micropipet (1 mm diameter) using the cerebral aqueduct and decussation of the superior cerebellar peduncle as visual landmarks. For measurement of adenylyl cyclase activity and phosphoinositide hydrolysis, a square section containing the dorsal raphe nucleus was removed from a unfrozen section using the above landmarks. Histological verification was performed by fixing sections in 4% paraformaldehyde/2.4% glutaraldehyde and then staining 50 μm sections with cresyl violet. The hippocampus was dissected from the remaining brain block according to Glowinski and Iversen (1966).

2.2. [^{3}H]8-OH-DPAT binding

The hippocampus and dorsal raphe nucleus core samples were homogenized in 50 mM Tris-HCl (pH 7.4) using a Dounce homogenizer (pestle B) and a microfuge tube with a Teflon pestle, respectively, and tissue homogenates were centrifuged at $40\,000 \times g$ for 20 min at 4°C. The resulting pellets were resuspended in the Tris buffer, and the samples were incubated at 37°C for 10 min to remove endogenous 5-HT (Nelson et al., 1978). Samples again were centrifuged at $40\,000 \times g$ for 20 min, and the final pellets were resuspended for the binding assays (dorsal raphe nucleus: 5.8 mg wet weight/ml; hippocampus: 30 mg wet weight/ml) in 50 mM Tris-HCl (pH 7.4), containing 10 μM clorgyline and 0.1% ascorbic acid. The [^{3}H]8-OH-DPAT binding assay was carried out using a modification of a previously described method (Winter and Rabin, 1992). Briefly, incubations were carried out for either 45 min at 25°C (dorsal raphe nucleus) or 30 min at 37°C (hippocampus) in a final volume of 300 μl containing 50 mM Tris-HCl (pH 7.4), [^{3}H]8-OH-DPAT (1 nM for competitions studies; 0.5–20 nM for equilibrium saturation studies), 10 μM clorgyline, 0.1% ascorbic acid and appropriate receptor ligands. Reactions were initiated by the addition of tissue homogenate (4.5 mg wet weight of hippocampus or 0.9 mg wet weight of dorsal raphe) and were terminated with a Brandell cell harvester. The Whatman GF/B filters were rinsed twice with cold 50 mM Tris-HCl (pH 7.4), and the amount of bound radioactivity was measured by liquid scintillation spectrophotometry after incubating the filters in scintillation cocktail overnight. Although equilibrium binding in the dorsal raphe nucleus could not be obtained at 30°C or 37°C, initial studies demonstrated that the assay conditions used for each tissue resulted in equilibrium binding. Specific binding was defined as the difference in the amount of radioactivity bound in the absence and presence of 10 μM 5-HT. The

data were analyzed by nonlinear regression using the program EBDA/LIGAND (Elsevier BIOSOFT, Cambridge, UK).

Protein content was determined by the method of Lowry et al. (1951) using bovine serum albumin (fraction V) as the standard.

2.3. Adenylyl cyclase assay

Tissues were homogenized in ice-cold 2 mM Tris-HCl (pH 7.5) containing 2 mM EGTA, 300 mM sucrose and 2 mM dithiothreitol, and the homogenates were centrifuged at 40 000 × *g* for 20 min at 4°C. The resulting pellets were resuspended in the Tris buffer, and the samples again were centrifuged at 40 000 × *g* for 20 min at 4°C. This procedure was repeated and the final pellet was resuspended in the Tris buffer. Adenylyl cyclase assays were carried out at 30°C in a final volume of 100 μl containing 50 mM Tris-HCl (pH 7.5), 5 mM cAMP, 2 mM $MgCl_2$, 0.1 mM ATP, 1–2 μCi of [α-^{32}P]ATP, 4 mM theophylline, 10 μM clorgyline, 10 mM creatine phosphate, 0.1 mg/ml creatine phosphokinase, 50 μM GTP, 100 mM NaCl and appropriate drugs. The reaction was stopped after 10 min by the addition of 100 μl of 50 mM Tris-HCl (pH 7.5) containing 5 mM ATP and 10% SDS. The samples were then boiled for 5 min, and [^{3}H]cAMP (approx. 15 000 cpm) was added to monitor recovery. [^{32}P]cAMP was isolated by sequential chromatography on Dowex 50 cation exchanger and neutral alumina as described by Rabin and Molinoff (1983).

2.4. Phosphoinositide hydrolysis

Phosphoinositide hydrolysis was determined using a modification (Fiorella et al., 1995) of the method of Conn and Sanders-Bush (1986). Dorsal raphe nucleus and hippocampus were cross-chopped (McIlwain tissue chopper) into 0.35 × 0.35 mm slices, and the slices were incubated for 30 min at 37°C in calcium-free Krebs-Henseleit bicarbonate saline (KHBS) containing 126 mM NaCl, 3 mM KCl, 1.4 mM KH_2PO_4, 1.3 mM $MgSO_4$, 25 mM $NaHCO_3$ and 11 mM glucose (pH 7.4) that was oxygenated with 95% O_2-5% CO_2. Medium was replaced with fresh, oxygenated buffer every 5 min during this preincubation. Slices were labeled by a 60 min incubation at 37°C in oxygenated, modified KHBS buffer (113 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 0.6 mM $MgSO_4$, 25 mM $NaHCO_3$ and 11.5 mM glucose) containing 10 μCi [^{3}H]myoinositol. After labeling, the tissue slices were washed 4 times with the modified KHBS buffer containing 10 mM unlabeled myoinositol and then incubated for 2 min in the modified KHBS with 10 mM LiCl and 5 mM myoinositol. Assays were carried out by incubating the tissue slices (25 μl of gravity-packed slices) for 45 min at 37°C in a final volume of 300 μl of modified KHBS buffer containing 10 mM LiCl, 10 μM clorgyline and various ligands. Reactions were stopped by the addition of 3 volumes of an ice-cold chloroform/methanol (1:2, v/v) solution followed by additional chloroform and water. After separating the phases by centrifuged at 1500 × *g* for 10 min, an aliquot of the upper aqueous phase was applied to a Dowex-1 anionic-exchange resin and the [^{3}H]inositol phosphates were eluted as described by Brown et al. (1984). Total amount of [^{3}H]myoinositol incorporated into the membrane lipids was quantified by washing the organic layer thrice with 5 mM myoinositol and measuring the incorporated radioactivity by scintillation spectroscopy. The total amount of [^{3}H]inositol phosphates generated (i.e., sum of [^{3}H]inositol 1-phosphate + [^{3}H]inositol 1,4-bisphosphate + [^{3}H]inositol 1,4,5-trisphosphate) was normalized to the amount of [^{3}H]myoinositol incorporated.

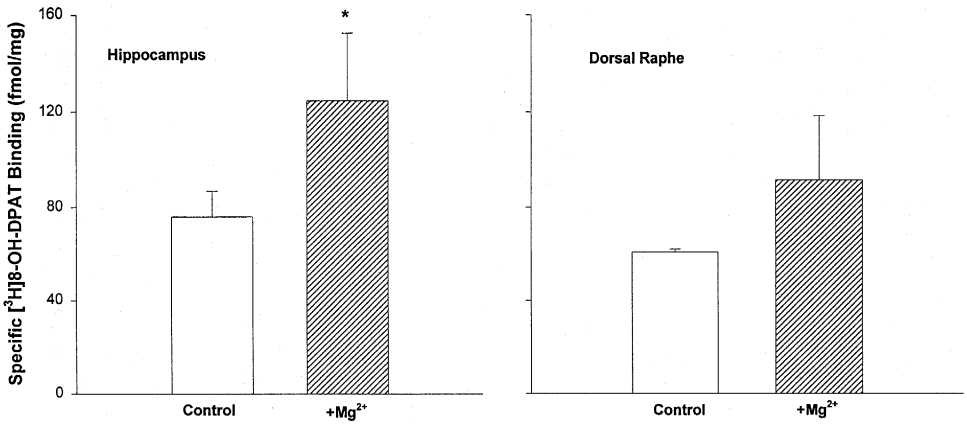

Fig. 1. Effect of Mg^{2+} on specific binding of [^{3}H]8-OH-DPAT to dorsal raphe and hippocampal membranes. Membranes from the hippocampus (left) and the dorsal raphe nucleus (right) were incubated with 2 nM [^{3}H]8-OH-DPAT in the absence or presence of 1 mM $MgCl_2$. The data are plotted as mean ± S.E.M. of three separate experiments. For each experiment, dorsal raphe nucleus and hippocampus were taken from the same animals. $^*P < 0.05$ (paired *t*-test) compared to appropriate control.

2.5. Materials

[^{3}H]8-OH-DPAT), [α-^{32}P]ATP and [^{3}H]cAMP were obtained from Dupont/New England Nuclear Research Products (Boston, MA, USA). [^{3}H]Myoinositol was obtained from American Radiolabeled Chemicals (St. Louis, MO, USA). All other chemicals were obtained from commercial sources.

3. Results

In agreement with previous reports (Norman et al., 1985; Hall et al., 1985, 1986; Emerit et al., 1990) using hippocampus, the presence of 1 mM Mg^{2+} significantly increased specific [^{3}H]8-OH-DPAT binding 62 ± 7% (Fig. 1), while addition of 1 mM GTP decreased radioligand binding by 57 ± 2% (Fig. 2). In the dorsal raphe nucleus, GTP decreased [^{3}H]8-OH-DPAT binding 42 ± 6%, while inclusion of Mg^{2+} tended to enhance specific binding; this increase was not statistically significant ($P = 0.1796$; Fig. 1). In both tissues inclusion of Mg^{2+} did not alter non-specific binding and thus, 1 mM $MgCl_2$ was included in all assays. Analysis of equilibrium saturation experiments indicated [^{3}H]8-OH-DPAT bound to a single population of sites in both the dorsal raphe nucleus and hippocampus (Fig. 3; Hill coefficient was 1.00 ± 0.02 and 1.05 ± 0.04 for dorsal raphe nucleus and hippocampus, respectively). However, the hippocampal site had a significantly higher affinity for the radioligand (pK_d was 8.84 ± 0.078 and 8.10 ± 0.074 for the hippocampus and dorsal raphe nucleus, respectively; $P < 0.01$; $n = 3$). The density of [^{3}H]8-OH-DPAT binding sites was 258 ± 23 fmol/mg and 891 ± 273 fmol/mg in the hippocampus and dorsal raphe nucleus, respectively. The [^{3}H]8-OH-DPAT binding site in the dorsal raphe nucleus displayed reasonable affinity for 5-HT and selective 5-HT$_{1A}$ receptor ligands such as flesinoxan, buspirone and gepirone (Table 1). Dopamine, epinephrine and phenylephrine did not displace [^{3}H]8-

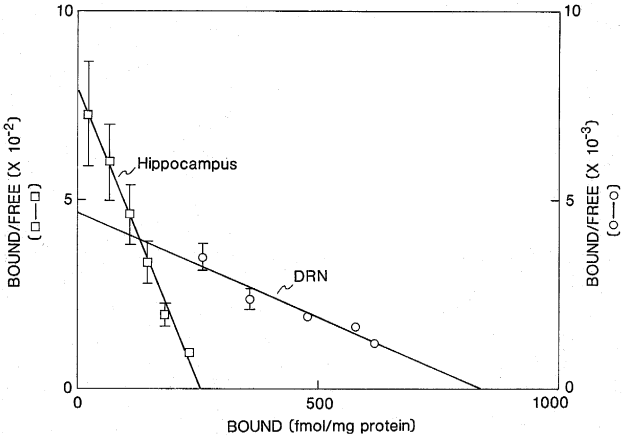

Fig. 3. Scatchard plot of equilibrium saturation binding of [^{3}H]8-OH-DPAT. Equilibrium saturation binding studies were carried out using 0.5–20 nM [^{3}H]8-OH-DPAT. Results are plotted by the method of Scatchard (1949) and are means ± S.E.M. of 3 experiments. For each experiment dorsal raphe membranes (○) and hippocampal membranes (□) were taken from the same animal.

Table 1
Pharmacological profile of the [^{3}H]8-OH-DPAT site in the dorsal raphe nucleus and hippocampus

	K_i (nM)	
	Dorsal raphe nucleus	Hippocampus
MDL 73005.EF	10.5 ± 2.5	3.9 ± 0.2
5-HT	10.7 ± 1.3	N.D.
Flesinoxan	13 ± 8	2.1 ± 0.9
Buspirone	72 ± 2	30 ± 5
L657.743	120 ± 25	32 ± 7
Yohimbine	151 ± 28	85 ± 10
Gepirone	164 ± 37	90 ± 7
MJ 14802	658 ± 124	130 ± 21

Hippocampal and dorsal raphe membranes were incubated in the presence of 2 nM [^{3}H]8-OH-DPAT and various concentrations of unlabeled receptor ligands as described in Section 2. Equilibrium dissociation constants for the unlabeled ligands (K_i) were determined using nonlinear regression analysis and are reported as mean ± S.E.M. for 3 separate experiments. N.D., not determined.

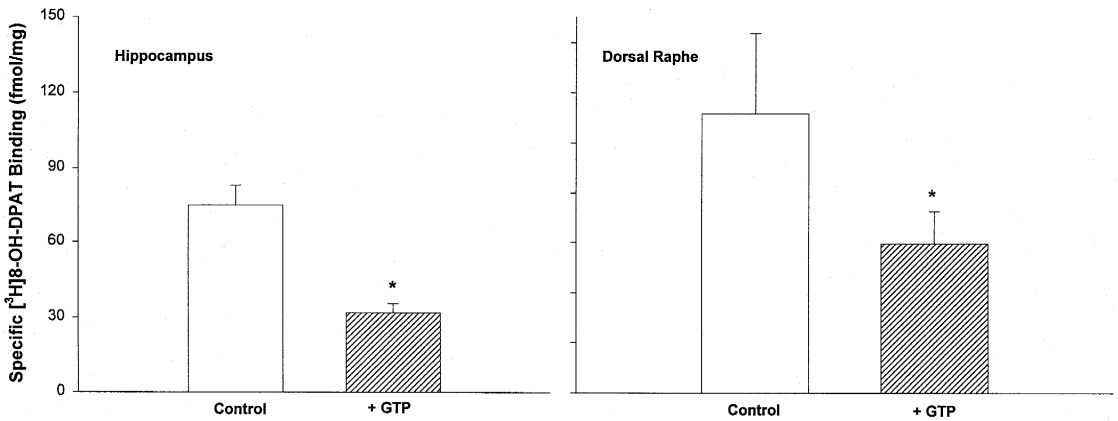

Fig. 2. Effect of GTP on specific binding of [^{3}H]8-OH-DPAT to dorsal raphe and hippocampal membranes. Membranes from the hippocampus (left) and the dorsal raphe nucleus (right) were incubated with 1 nM [^{3}H]8-OH-DPAT in the absence or presence of 1 mM GTP. The data are plotted as mean ± S.E.M. of three separate experiments. For each experiment, dorsal raphe nucleus and hippocampus were taken from the same animals. $^*P < 0.05$ (paired *t*-test) compared to the appropriate control.

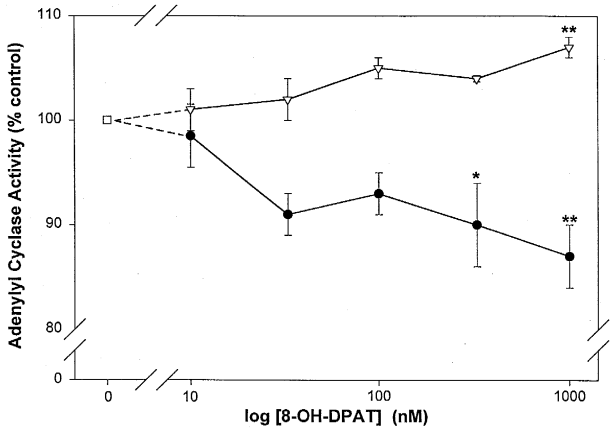

Fig. 4. Effect of 8-OH-DPAT on forskolin-stimulated adenylyl cyclase activity in the hippocampus and dorsal raphe nucleus. The effects of various concentrations of 8-OH-DPAT on adenylyl cyclase activity in the dorsal raphe nucleus (▽) and hippocampus (●) were measured in the presence of 10 μM forskolin. Data are expressed as a percent of control, which represents forskolin-stimulated enzyme activity alone (1.07 ± 0.292 and 1.08 ± 0.162 nmol cAMP/10 min per mg protein in dorsal raphe and hippocampal tissues, respectively), and are plotted as the mean ± S.E.M. of 4 (dorsal raphe nucleus) and 7 (hippocampus) experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ (Dunnett's a posteriori analysis) compared to the appropriate control.

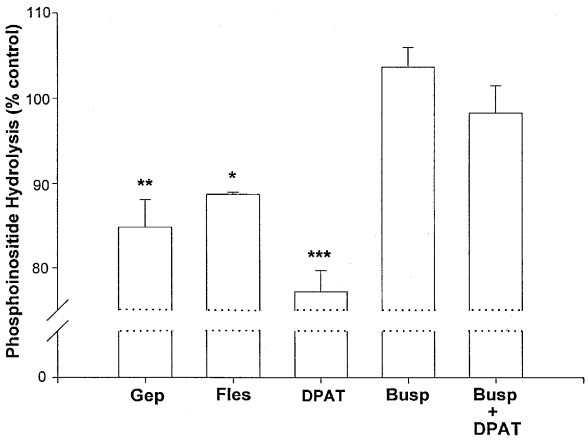

Fig. 6. Effect of various 5-HT_{1A} receptor ligands on phosphoinositide hydrolysis in the dorsal raphe nucleus. [^{3}H]Inositol phosphate accumulation in response to the 5-HT_{1A} receptor ligands, 8-OH-DPAT (DPAT), buspirone (Busp), gepirone (Gep) and flesinoxan (Fles), was determined in dorsal raphe slices. Data are expressed as a percent of control, which represents the normalized amount of [^{3}H]inositol phosphates generated in the absence of the 5-HT_{1A} receptor ligands and are plotted as mean ± S.E.M. of 3–7 separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (Tukey's a posteriori analysis) compared to the control response.

OH-DPAT from its binding site ($IC_{50} > 20$ μM, data not shown). Interestingly, the affinities of all the serotonergic receptor ligands were lower at the dorsal raphe site as compared to the hippocampal 5-HT_{1A} receptor.

In the dorsal raphe nucleus 8-OH-DPAT had no effect on basal adenylyl cyclase activity (13.35 ± 2.00 and 13.80 ± 1.48 pmol cAMP/min per mg protein in the absence and presence of 330 μM 8-OH-DPAT, respectively, $n = 3$).

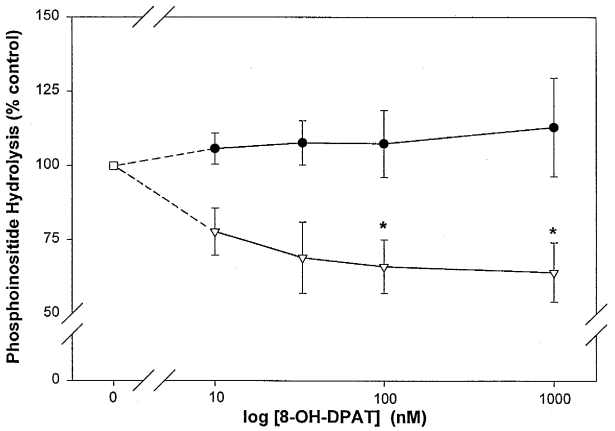

Fig. 5. Effect of 8-OH-DPAT on phosphoinositide hydrolysis in the hippocampus and dorsal raphe nucleus. Effects of various concentrations of 8-OH-DPAT on [^{3}H]inositol phosphate accumulation were measured in the hippocampus (●) and the dorsal raphe nucleus (▽). Data are expressed as a percent of control, which is the normalized amount of [^{3}H]inositol phosphates generated in the absence of 8-OH-DPAT in each tissue and are plotted as mean ± S.E.M. of 5 separate experiments. $^{*}P < 0.05$ (Dunnett's a posteriori analysis) compared to the appropriate control.

A concentration-dependent inhibition of forskolin-stimulated adenylyl cyclase activity was observed with 8-OH-DPAT in the hippocampus (Fig. 4). Conversely, 8-OH-DPAT did not inhibit forskolin stimulated enzyme activity in the dorsal raphe nucleus (Fig. 4).

Phosphoinositide hydrolysis in the dorsal raphe nucleus was inhibited in a concentration-dependent manner by the addition of 8-OH-DPAT (Fig. 5). Maximal inhibition of [^{3}H]inositol phosphate generation by 8-OH-DPAT was $45.5 \pm 11.3\%$ and the EC_{50} was 14.0 ± 4.04 nM ($n = 3$). Besides 8-OH-DPAT, the 5-HT_{1A} receptor selective ligands, gepirone and flesinoxan, also inhibited phosphoinositide hydrolysis in the dorsal raphe nucleus (Fig. 6). However, these ligands appeared to be acting as partial agonists inhibiting generation in the dorsal raphe nucleus by 13.8% and 11.3%, respectively (Fig. 6). The pyrimidinylpiperazine, buspirone, did not alter basal phosphoinositide hydrolysis. Rather, this compound completely antagonized the inhibition of [^{3}H]inositol phosphate accumulation by 8-OH-DPAT (Fig. 6). 8-OH-DPAT had no effect on phosphoinositide hydrolysis in the hippocampus (Fig. 5).

4. Discussion

Unlike the hippocampal 5-HT_{1A} receptor the [^{3}H]8-OH-DPAT site in the dorsal raphe nucleus is not coupled to adenylyl cyclase activity, but rather appears to be coupled to phosphoinositide turnover. Thus, in the dorsal raphe nucleus 8-OH-DPAT neither altered basal adenylyl cyclase activity nor did it inhibit forskolin-stimulated enzyme activity. This absence of 5-HT_{1A}-mediated inhibition

of adenylyl cyclase in the dorsal raphe nucleus cannot be explained by lack of receptor reserve as such a reserve exists in the dorsal raphe nucleus and not the hippocampus (Meller et al., 1990; Yocca et al., 1992). A similar inability of 5-HT_{1A} receptor agonists to inhibit forskolin-stimulated adenylyl cyclase activity in the dorsal raphe nucleus has recently been reported (Clarke et al., 1996).

The ability of the 5-HT_{1A} receptor agonists, 8-OH-DPAT, gepirone and flesinoxan, to inhibit basal phosphoinositide hydrolysis suggests that the [^{3}H]8-OH-DPAT site in the dorsal raphe nucleus is negatively coupled to phospholipase C. Similarly, activation of adenosine A_1 receptors has been reported to inhibit phosphoinositide turnover in a variety of tissues by reducing phospholipase C activity (see Linden and Delahunty, 1989). Although in the present study no such inhibitory effect was observed in the hippocampus, Claustre et al. (1988) reported that in the hippocampus the 5-HT_{1A} receptor agonists, 8-OH-DPAT, ipsapirone, gepirone and buspirone, inhibited carbachol-stimulated phosphoinositide hydrolysis. This inhibitory response, however, was specific for the muscarinic acetylcholine receptor as neither basal nor phosphoinositide turnover stimulated by KCl, quisqualate or norepinephrine were affected. In addition, these investigators subsequently showed that this inhibitory effect on hippocampal phosphoinositide hydrolysis was not a direct effect on phospholipase C, but rather involved a phosphorylation of the muscarinic acetylcholine receptor (Claustre et al., 1991). The inhibition of phosphoinositide hydrolysis in the dorsal raphe nucleus is not the result of cross-talk with the cyclic AMP system as 8-OH-DPAT did not alter adenylyl cyclase activity in the dorsal raphe nucleus. It is also unlikely that the inhibition of phosphoinositide hydrolysis in the dorsal raphe nucleus is secondary to a 5-HT_{1A}-mediated increase in K^+ conductance with a subsequent reduction in intracellular calcium as buspirone also increases K^+ conductance (Andrade and Nicoll, 1987), but did not inhibit phosphoinositide turnover.

In the hippocampus [^{3}H]8-OH-DPAT labels a 5-HT_{1A} receptor that is coupled through a pertussis toxin-sensitive G protein to both an inhibition of adenylyl cyclase activity and an increase in K^+ conductance (De Vivo and Maayani, 1986; Andrade et al., 1986). The [^{3}H]8-OH-DPAT binding site in the dorsal raphe nucleus displays the pharmacology consistent with a 5-HT receptor rather than an adrenergic or dopaminergic receptor and also appears to be coupled to a G protein as indicated by the response to Mg^{2+} and GTP. Because an identical rank order of potency was found for a series of 5-HT_{1A} receptor selective ligands in the hippocampus and dorsal raphe nucleus, the above data are consistent with [^{3}H]8-OH-DPAT also labeling a 5-HT_{1A} receptor in the dorsal raphe nucleus. Although 8-OH-DPAT has been reported to bind to the 5-HT transporter (Sprouse et al., 1993) and the 5-HT_7 receptor subtype (Ruat et al., 1993), the affinity of these sites for 8-OH-DPAT is much lower than that observed for [^{3}H]8-OH-DPAT binding in the dorsal raphe nucleus. In addition, the GTP and Mg^{2+} sensitivity of the dorsal raphe site is not consistent with the labeling of a 5-HT transporter. Also, unlike the [^{3}H]8-OH-DPAT binding site in the dorsal raphe nucleus, the 5-HT_7 receptor is positively coupled to adenylyl cyclase (Ruat et al., 1993). However, the site in the dorsal raphe nucleus has lower affinity for the 5-HT_{1A} receptor selective ligands than does the hippocampal 5-HT_{1A} receptor. Furthermore, the inhibitory effect of 8-OH-DPAT, gepirone and flesinoxan on phosphoinositide hydrolysis does not appear to fit the pharmacological profile of a 5-HT_{1A} receptor as buspirone, which also is a 5-HT_{1A} receptor agonist, behaved as a full antagonist displaying no efficacy but blocking the inhibitory action of 8-OH-DPAT. The lower affinity in the dorsal raphe nucleus cannot be explained by the difference in receptor reserve, and in fact is opposite to what would be predicted based on the observation that a significant receptor reserve exists in the dorsal raphe nucleus but not in the hippocampus (Meller et al., 1990; Yocca et al., 1992). The differences between the two tissues, however, could be explained by the 5-HT_{1A} receptor coupling to different G proteins. The 5-HT_{1A} receptor has been shown to couple with various signaling pathways in reconstituted systems by interacting with various G proteins (Fargin et al., 1989; Boddeke et al., 1992; Raymond et al., 1993). The specificity of this interaction appears to depend on the stoichiometry of the different G proteins and the 5-HT_{1A} receptor (Raymond et al., 1993). Alternatively, as three isoforms of the 5-HT_{1A} mRNA have been isolated (Albert et al., 1990), the differences between the dorsal raphe nucleus and hippocampus could be due to each tissue expressing a different mRNA isoform.

Acknowledgements

This work was supported by U.S. Public Service Grant DA03385 (to J.C.W. and R.A.R) and by a Mark Diamond Research grant awarded to R.G.J.

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