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5-HT_{1A} receptor-mediated regulation of mitogen-activated protein kinase phosphorylation in rat brain

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Abstract

Mitogen-activated protein kinases (MAPKs), a family of signal transduction mediators important in a host of cellular activities, include the extracellular signal-regulated kinases Erk1 and Erk2. We determined whether 5-HT_{1A} receptors activate Erk1/2 in rat brain in vivo, as they do in recombinant cell lines. In contrast to the effect in cells, the 5-HT_{1A} receptor agonist 8-hydroxy-*N*,*N*-diproylaminotetralin (8-OH-DPAT) dose- and time-dependently *decreased* basal levels of phosphorylated Erk1/2 (phospho-Erk1/2) in rat hippocampus (ED_{50} ~ 0.1 mg/kg, maximum ~ 90%) without altering total Erk1/2. The effects were kinase-specific, as 8-OH-DPAT did not modify phosphorylated or total levels of the MAPKs c-Jun-N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 MAPK. Moreover, 8-OH-DPAT did not modify phospho-Erk1/2 in striatum or frontal cortex. The effect of 8-OH-DPAT was blocked by pretreatment with the selective 5-HT_{1A} receptor antagonists *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY 100,635), 1-(2-methoxyphenyl)-4-(4-[2-phthalimido]butyl)piperazine (NAN-190) and 4-fluoro-*N*-(2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-*N*-(2-pyridinyl)benzamide dihydrochloride (p-MPPF), but not by the weak partial agonist/antagonist 8-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-8-azaspiro(4.5)decane-7,9-dione dihydrochloride (BMY 7378). Other 5-HT_{1A} receptor agonists (buspirone, gepirone and ipsapirone) also reduced phospho-Erk1/2 levels in hippocampus. 8-OH-DPAT also reduced the levels of the upstream activator of Erk1/2, phosphorylated extracellular signal-regulated kinase kinase (phospho-MEK1/2), and at least one potential downstream target, the nuclear transcription factor phospho-Elk-1. The region- and kinase-specific effects suggest that the Erk1/2 signal transduction cascade is likely an important differential mediator of 5-HT_{1A} receptor-regulated events in the central nervous system.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Hippocampus; 5-HT_{1A} receptor; Phosphorylation; Erk1/2; MAP (mitogen-activated protein) kinase; Immunoblotting

1. Introduction

Of the approximately 15 5-HT receptor subtypes identified to date, the 5-HT_{1A} receptor subtype has arguably received the most attention. Primarily this is because alterations in 5-HT_{1A} receptor function have been implicated in anxiety and depression (De Vry et al., 1991; Heisler et al., 1998), but this receptor also plays important roles in aggression (Korte et al., 1996), neuroendocrine function (Meller and Bohmaker, 1994; Li et al., 1996), temperature regulation (Meller et al., 1992; Millan et al., 1993), sexual behavior (Uphouse et al., 1991) and food intake (Ebenezer et al., 1999). The 5-HT_{1A} receptor is a prototypical G-protein-coupled receptor, and its neuronal signaling pathways have been shown to include opening of K^+ channels (Andrade and Nicoll, 1987) and inhibition of adenylyl cyclase (De Vivo and Maayani, 1986), Ca^{2+} channels (Penington and Kelly, 1990) and phospholipase C activation (Claustre et al., 1991a). Virtually all of its effects are mediated via activation of pertussis toxin-sensitive (i.e., G_i and/or G_o) G proteins. Activation of many G-protein-coupled receptors, however, results in cross-talk with receptor tyrosine kinase signaling pathways, such as the mitogen-activated protein (MAP) kinases Erk1 and Erk2 (p44 and p42MAPK, respectively) (Gutkind, 1998). Indeed, it has been shown that the recombinant 5-HT_{1A} receptor is linked to multiple signaling pathways (Raymond et al., 1999), including activation of Erk1/2 (Garnovskaya et al., 1996,

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1998; Mukhin et al., 2000), and that the specific pathway depends on the host cell in which the receptor is expressed (Raymond et al., 1999). Both 5-HT_{1A} receptors (Pompeiano et al., 1992) and Erk1/2 (Flood et al., 1998) are highly expressed in the hippocampus, and activation of hippocampal Erk1/2 has been shown to occur after various stimuli, including seizure induction (Murray et al., 1998), ischemic insult (Alessandrini et al., 1999; Hicks et al., 2000), electroconvulsive shock (Kang et al., 1994; Bhat et al., 1998), induction of long-term potentiation (English and Sweatt, 1997; Davis et al., 2000) and avoidance learning (Atkins et al., 1998; Cammarota et al., 2000). It seemed reasonable, therefore, to determine if native 5-HT_{1A} receptors activate the MAPK pathway in vivo. We therefore investigated the effect of acute systemic treatment with the 5-HT_{1A} receptor agonist 8-hydroxy-dipropylaminotetralin (8-OH-DPAT) on the phosphorylation state of the three major MAP kinases, Erk1/2, c-Jun-N-terminal kinase/stress-activated protein kinase (JNK/SAPK) and p38 MAPK, in the rat hippocampus.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (175–200 g; Taconic Farms, Germantown, NY) were used in all experiments. They were maintained on a 12-h light/dark cycle and housed two per cage with food and water ad libitum. All experimental procedures were carried out in accordance with the U.S. NIH Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee.

2.2. Drug treatments

All drugs were obtained from Sigma/RBI (St. Louis, MO), except as noted. Rats were treated acutely, intraperitoneally (i.p.) or subcutaneously (s.c.), with saline vehicle or various drugs at the doses indicated in the figure legends. The following drugs were used: the 5-HT_{1A} receptor agonists 8-OH-DPAT, buspirone, gepirone (the latter two gifts of Bristol Myers-Squibb, Wallingford, CT), and ipsapirone (a gift of Miles, West Haven, CT), and the 5-HT_{1A} receptor antagonists *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY 100,635), 8-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-8-azaspiro(4.5)decane-7,9-dione dihydrochloride (BMY 7378), 1-(2-methoxyphenyl)-4-(4-[2-phthalimido]butyl)piperazine (NAN-190) and 4-fluoro-*N*-(2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl)-*N*-(2-pyridinyl)benzamide dihydrochloride (p-MPPF). Animals were sacrificed at various times after drug treatment, brains were removed and the hippocampus, striatum and frontal cortex were rapidly dissected and stored at −80 °C. In some experiments, groups of rats were administered the irreversible receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; Sigma) (6 mg/kg, s.c.) or its vehicle (ethanol/propylene glycol/water, 1:1:2). After 24 h, animals in each group were treated with saline or various doses of 8-OH-DPAT (0.01–3 mg/kg, s.c.), sacrificed 30 min later and the hippocampi were dissected and frozen.

2.3. Preparation of total tissue extracts

Tissues were homogenized in 10 volumes of 50 mM Tris–HCl, pH 7.4 containing 300 mM NaCl, 1% Nonidet P-40, 10% glycerol, 1 mM EDTA, 1 mM Na_3VO_4, 1 mM NaF and protease inhibitor cocktail (Complete®, Roche Molecular Biochemicals, Indianapolis, IN). After centrifugation at 30,000 × *g* for 20 min, lysates were mixed with 2 × sodium dodecyl sulfate (SDS) sample buffer, boiled for 5 min and stored at −80 °C. Protein content of lysates was determined using the bicinchoninic acid assay kit (Pierce Chemical, Rockford, IL). All experiments were carried out using total tissue extracts unless noted otherwise.

2.4. Cytoplasmic and nuclear extracts

Cytosolic and nuclear extracts were prepared as described by Cammarota et al. (2000). Tissues were homogenized in 10 volumes of 20 mM Tris–HCl, pH 7.4, containing 0.32 M sucrose, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride and protease inhibitor cocktail (Complete®). After centrifugation at 900 × *g* for 10 min, the resulting supernatant was centrifuged at 30,000 × *g* for 20 min to yield cytosolic extract. The pellet from the low-speed spin was washed, loaded on a 2 M sucrose cushion and centrifuged (100,000 × *g*, 60 min). The supernatant and the sucrose cushion were discarded, and the pellet was homogenized in 20 mM Tris–HCl, pH 7.9, containing 12% glycerol, 0.5% Nonidet P-40, 500 mM NaCl, 50 mM NaF, 1 mM Na_3VO_4 and 1 mM phenylmethylsulfonyl fluoride. After end-over-end rotation for 30 min at 4 °C to extract nuclear proteins, the homogenate was centrifuged (32,000 × *g*, 20 min) and the supernatant was removed and dialyzed for 1 h (Pierce Slide-A-Lyzer). The cytosolic and nuclear extracts were mixed with sample buffer, boiled for 5 min and stored at −80 °C.

2.5. SDS-PAGE and immunoblotting

Proteins (5–15 μg/lane) were separated by electrophoresis on precast 10% polyacrylamide gels (ICN, Aurora, OH). Lanes on each gel were also loaded with colored molecular weight markers (Bio-Rad, Hercules, CA) to assess completeness of electrophoretic transfer, and Cruz Marker molecular weight standards (Santa Cruz) to estimate the size of bands of interest. After electrophoretic transfer to nitrocellulose sheets, the blots were incubated in blocking buffer (3% nonfat dry milk in Tris-buffered saline contain-

ing 0.05% Tween-20 (TBST)) for 1 h at room temperature, washed 3 × 10 min in TBST and incubated with primary phospho-specific antibodies overnight at 4 °C (monoclonal antibodies in 3% milk/TBST, polyclonal antibodies in 5% bovine serum albumin/TBST). Blots were washed 3 × in TBST, incubated with the appropriate secondary antibody for 1 h at room temperature, washed again, treated with West Pico enhanced chemiluminescence (ECL) reagent (Pierce) and exposed to film. Blots were then stripped of antibodies by incubation for 15 min at room temperature with Re-Blot Plus (Chemicon, Temecula, CA), re-blocked, washed and probed for 1 h at room temperature with the corresponding antibody that recognizes total antigen protein (phosphorylated and nonphosphorylated), followed by incubation for 1 h at room temperature with the appropriate secondary antibody. Antigens were again visualized by treatment with ECL reagent and exposure to film. The following primary antibodies were used at the indicated dilutions: phospho-Erk (E-4 monoclonal, 1:4000–1:8000), Erk1 (K-23 rabbit polyclonal, 1:8000–1:16,000), mitogen-activated protein kinase phosphatase-1 (MKP-1, V-15 rabbit polyclonal, 1:1000) and MKP-3 (C-20, goat polyclonal, 1:1000) were from Santa Cruz Biotechnology (Santa Cruz, CA); all other primary antibodies were rabbit polyclonal from Cell Signaling Technology (Beverly, MA) unless indicated otherwise (phospho-JNK, G-9 monoclonal, 1:1000; JNK, 1:1000; phospho-p38 MAPK, 1:1000; p38 MAPK, 1:1000; phospho-MEK1/2, 1:3000; MEK1/2, 1:4000; phospho-Elk-1, 2B1 monoclonal 1:1000). Secondary antibodies (Santa Cruz) were always used at twice the dilution of primary antibodies. All immunoblots shown are representative of at least three separate experiments with similar results.

2.6. Quantitative immunoblot analysis

Immunoblots were analyzed using a computerized image analysis system (MCID-M4, Imaging Research, St. Catherines, Ontario, Canada). Films were exposed and developed such that the maximum absorbance never exceeded 1.0, which was well within the linear range of the film exposure–response curve as previously determined (Meller et al., 2000). For each blot, relative phospho-Erk2 levels were calculated after normalization for small differences in protein loading by taking the ratio of absorbance of phospho-Erk2/total Erk; changes for each experimental condition were obtained by comparison to the control ratio.

2.7. Statistical analysis

Dose–response curves (vehicle and EEDQ-pretreated) were simultaneously analyzed for best fit using the ALLFIT-PC computer program (Dr. Peter Munson, NICHHD, Bethesda, MD). Its use has been described previously in detail (De Lean et al., 1978; Meller et al., 1990, 1991). The program tests the goodness of fit after curves are constrained to share one or more parameters (response at zero dose, slope factor, ED_{50} and response at "infinite" dose). Curves were initially analyzed without constraints and then after sequentially constraining them to share a common slope factor, ED_{50} or maximal response. The analysis that allowed one or more of the parameters to be shared without a significant increase in the residual variance was considered the best fit (De Lean et al., 1978; Meller et al., 1990). Where appropriate, samples from control and treated rats were compared using Student's *t*-test (SigmaStat, ver. 2.03, SPPS, Chicago, IL).

3. Results

3.1. Dose- and time-dependent effects of 8-OH-DPAT on phospho-Erk2 levels in hippocampus

Acute treatment with 8-OH-DPAT (0.5 mg/kg) rapidly and profoundly reduced phospho-Erk2 (p42MAPK) levels in the hippocampus, with a maximal effect (>90% inhibition) at 30 min, which began to return to normal by 60–120 min (Fig. 1, top). In contrast, total Erk2 levels were essentially unchanged. Although it was difficult to quantitate phospho-Erk1 (p44MAPK) levels in hippocampus due to its low abundance relative to phospho-Erk2, it was obvious from overexposed blots that, qualitatively, 8-OH-DPAT treatment had a similar effect in reducing phospho-Erk1 levels (data not shown). The effect of 8-OH-DPAT was clearly dose-dependent, and its ED_{50} was approximately 0.1 mg/kg (Fig. 1, bottom).

3.2. Lack of effect of 8-OH-DPAT on phospho-Erk2 levels in striatum and frontal cortex

In contrast to its marked effect in reducing phospho-Erk2 levels in the hippocampus, 8-OH-DPAT did not alter phos-

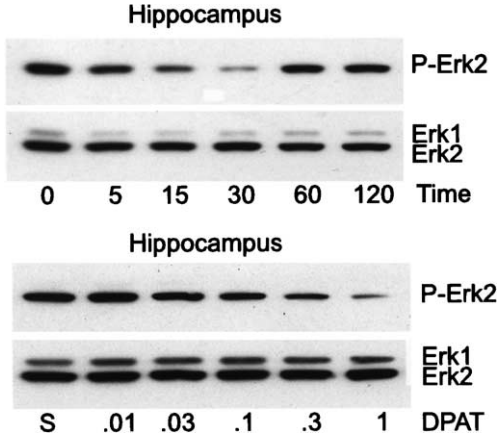

Fig. 1. Top: time-course (min) of 8-OH-DPAT (0.5 mg/kg, s.c.) effect on phospho-Erk2 and total Erk1/2 levels in hippocampus. Saline (S) was injected 30 min before sacrifice. Bottom: dose–response (mg/kg, s.c.) of 8-OH-DPAT treatment. P-Erk2, phospho-Erk2; DPAT, 8-OH-DPAT.

pho-Erk1/2 levels in either the frontal cortex (Fig. 2, top) or striatum (Fig. 2, bottom). While the latter effect was not unexpected, given the absence of 5-HT_{1A} receptors in the striatum (Pompeiano et al., 1992), the lack of an effect in the frontal cortex was somewhat surprising in view of the relative abundance of 5-HT_{1A} receptors in cortical areas (Pompeiano et al., 1992).

3.3. Lack of effect of 8-OH-DPAT on other phospho-MAPKs in hippocampus

Blots of hippocampal tissue extracts were also probed with specific antibodies to the other two major MAPK phosphoprotein families, JNK/SAPK and p38 MAPK (Graves and Krebs, 1999). Neither the 46- nor 54-kDa isoforms (JNK1 and JNK2, respectively) of phospho-JNK/SAPK (Fig. 3, top), nor totals levels of these proteins were altered by acute 8-OH-DPAT treatment. Similarly, neither phospho-p38 MAPK nor total p38 MAPK levels were changed by the drug (Fig. 3, bottom).

3.4. Blockade of 8-OH-DPAT-induced suppression of hippocampal phospho-Erk2 levels by 5-HT_{1A} receptor antagonists

Pretreatment with the selective 5-HT_{1A} receptor antagonist WAY 100,635 (0.1 mg/kg) (Forster et al., 1995) almost completely blocked the inhibitory effect of 8-OH-DPAT on phospho-Erk2 levels in hippocampus (Fig. 4, top). In this experiment, WAY 100,635 by itself appeared to increase phospho-Erk2 levels; however, this effect was not observed in other repetitions of this study (data not shown). Other 5-HT_{1A} receptor antagonists, including NAN-190 (10 mg/kg) (Claustre et al., 1991b) and p-MPPF (5 mg/kg) (Kung et al., 1996), also partially blocked the effect of 8-OH-DPAT (Fig. 4, bottom; 80–85% of control, $n=4$, not significantly different from control); in contrast, the partial agonist/antagonist BMY 7378 (20 mg/kg) (Meller et al., 1990) did not attenuate the suppressive effect of the agonist (saline plus 8-OH-DPAT, 62 ± 4% of control ($n=4$); BMY 7378 plus 8-OH-DPAT, 42 ± 3% of control ($n=4$); each $P<0.05$ vs. saline plus saline).

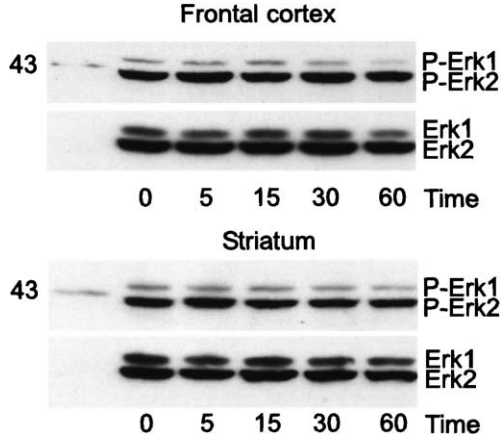

Fig. 2. Phospho- and total Erk1/2 levels in frontal cortex and striatum at various times after treatment with 8-OH-DPAT (0.5 mg/kg). In this and subsequent figures, protein molecular weight markers (in kDa) are shown on the left.

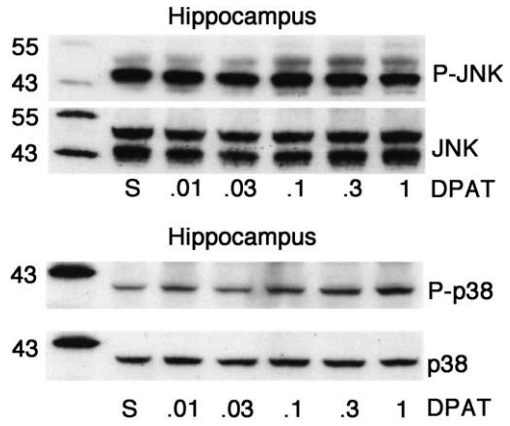

Fig. 3. Phospho- and total JNK and p38 MAPK levels in hippocampus 30 min after injection of various doses of 8-OH-DPAT (mg/kg).

3.5. Effects of other 5-HT_{1A} receptor agonists

Similarly to 8-OH-DPAT, the 5-HT_{1A} receptor agonists buspirone, gepirone and ipsapirone all reduced phospho-Erk2 levels in hippocampus, to varying degrees (Fig. 5; mean ± S.E.M. percent of control, $n=3–5$: 8-OH-DPAT, 18 ± 5; buspirone, 43 ± 8; gepirone, 30 ± 5; ipsapirone, 57 ± 10; all $P<0.05$ vs. saline).

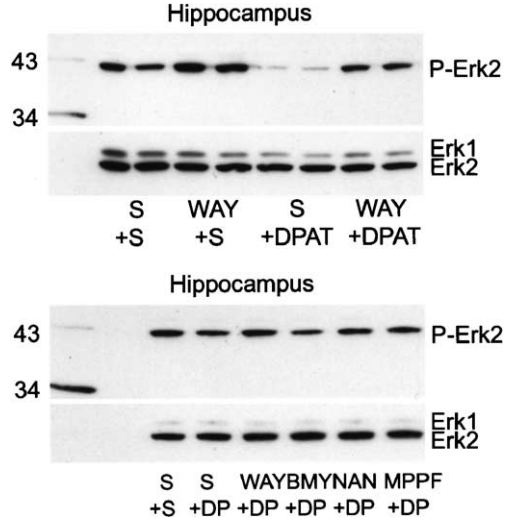

Fig. 4. Blockade of 8-OH-DPAT (0.5 mg/kg)-induced reduction of hippocampal phospho-Erk2 by pretreatment with 5-HT_{1A} receptor antagonists. WAY 100,635 (WAY; 0.1 mg/kg), BMY 7378 (BMY; 20 mg/kg), NAN-190 (NAN; 10 mg/kg), p-MPPF (MPPF; 5 mg/kg) or saline (S) was administered 30 min before 8-OH-DPAT (DPAT or DP; 0.5 mg/kg) or saline, and animals were sacrificed 30 min later.

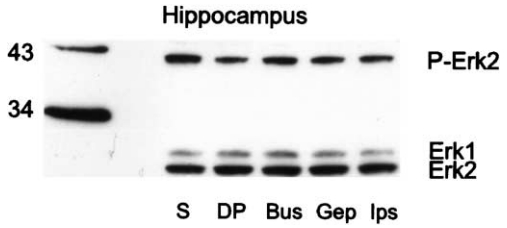

Fig. 5. Reduction in hippocampal phospho-Erk2 levels by the 5-HT_{1A} agonists 8-OH-DPAT (0.5 mg/kg), buspirone (Bus; 10 mg/kg), gepirone (Gep; 10 mg/kg) and ipsapirone (Ips; 5 mg/kg).

3.6. Effect of 8-OH-DPAT on the phosphorylation state of an upstream activator of Erk and a downstream target of phospho-Erk

The MAPK pathway(s) entails a well-established cascade of sequential phosphorylation and activation of a three-kinase MAPK module (Garrington and Johnson, 1999). The immediate upstream regulator of Erk1/2 activity is the mitogen- and extracellular signal-regulated kinase (MEK1/2; also known as mitogen-activated protein kinase kinase or MAPKK) (Graves and Krebs, 1999; Garrington and Johnson, 1999). Once activated, phospho-Erk1/2 can in turn activate a diverse group of nuclear transcription factors (Garrington and Johnson, 1999). It was of interest therefore to determine if 8-OH-DPAT treatment also decreased the levels of the activated upstream kinase phospho-MEK1/2 and a typical nuclear target, the transcription factor phospho-Elk-1. Indeed, 8-OH-DPAT reduced phospho-MEK1/2 levels in hippocampus without altering total MEK1/2 (Fig. 6, top). Phospho-Elk-1 levels could not be detected in whole hippocampal extracts; however, it was readily detectable in nuclear extracts, and 8-OH-DPAT elicited a time-dependent reduction in its levels (Fig. 6, bottom). Interestingly, phospho-Elk-1 levels in the nucleus were still decreased markedly at 60 min after drug treatment; in contrast, phospho-Erk2 levels in total cellular extracts had rebounded by 60 min to near control levels (Fig. 1, top).

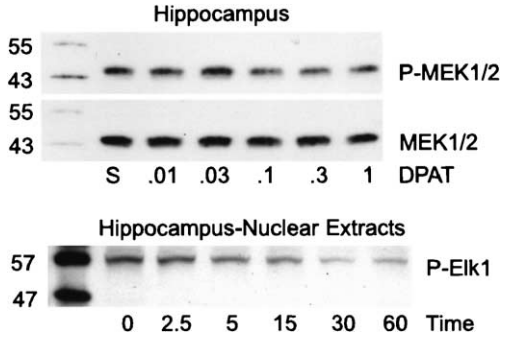

Fig. 6. Dose–response of 8-OH-DPAT-induced decrease of phospho-MEK1/2 in total cellular lysates (top), and 8-OH-DPAT (0.5 mg/kg) time-course of phospho-Elk-1 reduction in nuclear extracts (bottom).

3.7. Time-course of phospho-Erk2 decline in cytosolic and nuclear hippocampal extracts

Following its activation (phosphorylation) in the cytosolic compartment of cells, phospho-Erk is translocated to the nucleus, where it activates its target(s) (Graves and Krebs, 1999; Schaeffer and Weber, 1999). Since nuclear phospho-Elk-1 levels were clearly reduced by 8-OH-DPAT, the drug's effects on phospho-Erk2 concentrations in cytosolic and nuclear extracts were compared. Fig. 7 (top) demonstrates that the time-course of the 8-OH-DPAT-induced decrease in cytosolic phospho-Erk2 is similar to that found when total tissue extract was analyzed (cf. Fig. 1, top); in particular, note the maximal reduction at 30 min and the rebound toward control levels by 1 h. Nuclear extracts exhibited a similar time-course for phospho-Erk2 levels (Fig. 7, bottom), except that, as for phospho-Elk-1, there was no rebound at 1 h (see Discussion).

3.8. Effect of irreversible 5-HT_{1A} receptor inactivation on the 8-OH-DPAT dose–response curve

In order to distinguish whether the 5-HT_{1A} receptors mediating the in vivo effect of 8-OH-DPAT are presynaptic (i.e., somatodendritic autoreceptors in the raphe nuclei) or postsynaptic (in the hippocampus), dose–response curves were generated for 8-OH-DPAT suppression of hippocampal phospho-Erk2 before and after partial irreversible inactivation of 5-HT_{1A} receptors with EEDQ (Meller et al., 1990). We previously established that for autoreceptor-mediated responses, partial irreversible receptor inactivation produces a rightward shift in the ED_{50} (Meller et al., 1990; Cox et al., 1993), whereas for postsynaptic receptor responses, a decrease in maximal response without a shift in ED_{50} results (Yocca et al., 1992; Meller et al., 1992) (see Discussion). EEDQ treatment produced a large rightward shift in the ED_{50} for 8-OH-DPAT (6.7-fold) (Fig. 8), consistent with a somatodendritic autoreceptor localization of the 5-HT_{1A}

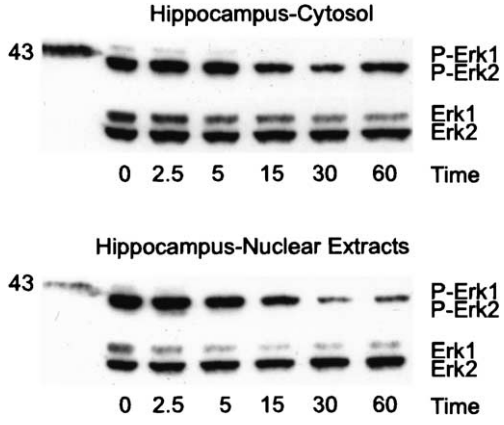

Fig. 7. 8-OH-DPAT (0.5 mg/kg)-elicited decreases in cytosolic and nuclear phospho-Erk2 as a function of time.

receptors mediating the agonist-induced reduction in hippocampal phospho-Erk2 levels.

4. Discussion

The major finding of the present study is that, whereas 5-HT_{1A} receptors in recombinant cell lines *activate* the Erk1/2 MAPK pathway, they *inhibit* this pathway in the rat hippocampus in vivo. The effect exhibited dose- and time dependency (Fig. 1), anatomical specificity (observed in hippocampus but not frontal cortex or striatum; Fig. 2) and kinase specificity (the Erk1/2 pathway but neither the JNK/SAPK nor the p38 MAPK was affected; Fig. 3). The effect was clearly 5-HT_{1A} receptor-mediated, as it was blocked by 5-HT_{1A} receptor antagonists (WAY 100,635, NAN-190, pMPPF) (Fig. 4) and reproduced by other 5-HT_{1A} receptor agonists (buspirone, gepirone, ipsapirone) (Fig. 5). Although 8-OH-DPAT also displays considerable affinity for 5-HT_7 receptors (Lovenberg et al., 1993), blockade of its effect by a low dose of WAY 100,635 (Fig. 4), which has greater than 1000-fold selectivity for 5-HT_{1A} (Forster et al., 1995) over 5-HT_7 (Thomas et al., 2000) receptors, renders 5-HT_7 receptor involvement highly unlikely.

The mechanism(s) by which 5-HT_{1A} receptor activation in vivo reduces phospho-Erk1/2 levels in hippocampus is unknown. We addressed two general alternatives in initial studies: interference with regulatory mechanisms that maintain phospho-Erk levels, or inhibition of the MAPK cascade. In preliminary experiments, because a number of protein kinase phosphatases have been implicated in regulation of phospho-Erk levels (Haneda et al., 1999), we focused on whether 8-OH-DPAT treatment altered mitogen-activated protein kinase phosphatase-1 (MKP-1) or MKP-3 levels in hippocampus. MKP-1 levels were not modified by 8-OH-DPAT while MKP-3 could not be detected on immunoblots (data not shown). On the other hand, 8-OH-DPAT reduced the levels of the upstream activator of Erk1/2, phospho-MEK1/2, as well as one possible downstream target, the nuclear transcription factor phospho-Elk-1 (Fig. 6), lending preliminary support to the concept that 8-OH-DPAT-induced reductions in phospho-Erk1/2 may be the result of inhibition of the MAPK pathway upstream of the MAPK module. Further studies addressing this issue are planned.

The discovery of a new 5-HT_{1A} receptor-mediated functional response generated two important and related questions: (1) which population of brain 5-HT_{1A} receptors (pre- vs. postsynaptic) mediates the effect, and (2) in which subcellular (cytoplasmic vs. nuclear) and anatomic (pre- vs. postsynaptic) compartments does the affected phospho-Erk reside?

With regard to the first question, 5-HT_{1A} receptors in the brain are known to be localized to the soma/dendrites of raphe nuclei 5-HT neuronal perikarya and to their postsynaptic targets in the forebrain (Vergé et al., 1985). We previously demonstrated that functional responses mediated by these anatomically distinct 5-HT_{1A} receptor populations may be distinguished using a relatively simple pharmacological technique to evaluate the efficiency of receptor/effector coupling: somatodendritic autoreceptors exhibit high efficiency receptor/effector coupling (Meller et al., 1990; Cox et al., 1993), whereas postsynaptic receptors do not (Yocca et al., 1992; Meller et al., 1992). Experimentally, responses mediated by somatodendritic autoreceptors display rightward shifts in dose–response curves after partial irreversible receptor inactivation, whereas responses regulated by postsynaptic receptors do not. Interestingly, application of this technique indicated that stimulation of somatodendritic rather than postsynaptic 5-HT_{1A} receptors is primarily driving the 8-OH-DPAT-induced decrease in hippocampal phospho-Erk1/2, because EEDQ treatment clearly and significantly right-shifted the ED_{50} for the agonist (Fig. 8). Some quantitative and qualitative observations further support this conclusion. First, the control 8-OH-DPAT ED_{50} for this response (58 μg/kg) is similar (but 2- to 4-fold higher) to that previously determined for a somatodendritic 5-HT_{1A} receptor-mediated response (inhibition of 5-HTP synthesis; 15–30 μg/kg) (Meller et al., 1990). Second, the ED_{50} shift calculated here after EEDQ treatment (6.7-fold) is very similar to that found previously (6.1-fold) (Meller et al., 1990). In addition, BMY 7378, by virtue of its very weak partial agonist/antagonist character, behaves as an antagonist at postsynaptic 5-HT_{1A} receptors (Yocca et al., 1987) and as a seeming agonist at somatodendritic autoreceptors (Meller et al., 1990). BMY 7378 did not attenuate 8-OH-DPAT-induced phospho-Erk2 inhibition (Fig. 4), which is therefore inconsistent with a postsynaptic 5-HT_{1A} receptor antagonist action. Moreover, it was found

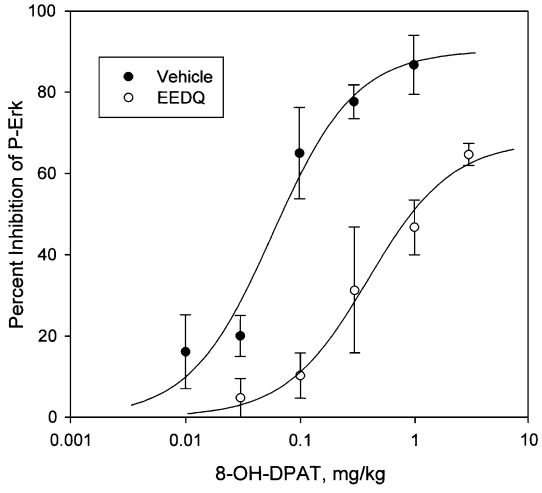

Fig. 8. Dose–response curves for 8-OH-DPAT-induced reduction in phospho-Erk2 levels in hippocampus of vehicle- and EEDQ (6 mg/kg)-pretreated rats. Dose–response curves were simultaneous analyzed with ALLFIT (De Lean et al., 1978), which indicated that the ED_{50} and maximal responses were significantly different: control, ED_{50} 58 μg/kg, maximum inhibition 91%; EEDQ, ED_{50} 394 μg/kg, maximum inhibition 68%. Each point is the mean ± S.E.M. of three determinations.

that BMY 7378 alone (20 mg/kg) inhibited phospho-Erk2 levels, consistent with an agonist effect at somatodendritic autoreceptors, although its efficacy appeared to be reduced relative to 8-OH-DPAT (data not shown). Overall, these results suggest that somatodendritic autoreceptor activation is the primary mediator of the response, but the data are not inconsistent with a partial contribution of postsynaptic receptor activation.

As concerns the second question, direct evidence to identify the subcellular and anatomic compartments of the phospho-Erk affected by 5-HT_{1A} receptor stimulation in vivo is not available. Much hippocampal Erk appears to be localized to the cytoplasm, in soma and dendrites (Bhat et al., 1998) (i.e., in postsynaptic cells). In cultured cells, stimulation with mitogens results in translocation of cytoplasmic phospho-Erk to the nucleus (Graves and Krebs, 1999; Schaeffer and Weber, 1999). There is also evidence for a *presynaptic* localization of phospho-Erk in neuronal terminals in the hippocampus (Murray et al., 1998). Since nuclear phospho-Erk2 (Fig. 7) and nuclear phospho-Elk-1 (Fig. 6) are both reduced by 8-OH-DPAT, it is clear that phospho-Erk2 in postsynaptic hippocampal cells is affected by 5-HT_{1A} receptor activation; however, it is not known whether this reflects a direct activation of postsynaptic hippocampal 5-HT_{1A} receptors, or indirect modification of postsynaptic cellular activity subsequent to activation of inhibitory somatodendritic autoreceptors and consequent inhibition of 5-HT neuronal input to the hippocampus. The 5-HT_{1A} autoreceptors appear to be primarily responsible for the reduction in phospho-Erk2 in total tissue extracts (Fig. 8). Unknown at this time is the relative contribution of presynaptic cytosolic, postsynaptic cytosolic and postsynaptic nuclear phospho-Erk to the total phospho-Erk immunoreactivity. It is likely that the nuclear contribution is small, and thus differential effects on this compartment may not be apparent when analyzing total extract. Indeed, as seen in Fig. 7, phospho-Erk2 levels in the cytosol begin to rebound at 60 min, similar to the effect in total extracts (Fig. 1), whereas in nuclear extracts, phospho-Erk2 levels are still markedly depressed at this time, analogous to the time-course of nuclear phospho-Elk-1 (Fig. 6). Whether this reflects a pre- vs. postsynaptic or cytosol vs. nuclear difference is unknown. It should be noted that these issues may prove difficult to resolve, as basal phospho-Erk1/2 levels, while easily measured on immunoblots, are almost undetectable immunohistochemically (Bhat et al., 1998; Murray et al., 1998), making it unlikely that drug-induced reductions will be discernable.

The most significant issue raised by the current results is what physiological function or activity underlies the reduction in Erk pathway activation in hippocampus after stimulation of 5-HT_{1A} receptors. Since this receptor appears to be importantly involved in anxiety/depression and many other vital functions (see Introduction), it will be of great interest to determine the extent to which the activity of the MAPK pathway is regulated in various models of 5-HT_{1A} receptor function.

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