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5-HT_{1A} receptors couple to activation of Akt, but not extracellular-regulated kinase (ERK), in cultured hippocampal neurons

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Abbreviations used:

BDNF	brain-derived neurotrophic factor
BrdU	bromodeoxyuridine
CHO	Chinese hamster ovary
5-CT	5-carboxamidotryptamine maleate
DAPI	4,6-diamino-2-phenolindol dihydrochloride
ERK	extracellular signal-regulated kinase
FITC	fluorescein isothiocyanate
GSK-3	glycogen synthase kinase-3
5-HT	5-hydroxytryptamine (serotonin)
MAP	microtubule-associated protein
8-OH-DPAT	(±)8-hydroxy-N,N-dipropyl-2-aminotetralin hydrobromide
PBS	phosphate-buffered saline
PDK	3-phosphoinositide-dependent kinase
PI3K	phosphatidylinositol 3-kinase
p-MPPI	4-Iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-benzamide hydrochloride
SB269970	[R]-3-[2-(2-[4-Methyl-piperidin-1-yl]ethyl)pyrrolidine-1-sulfonyl]phenol
SSRI	selective serotonin reuptake inhibitor
WAY-100635	N-[2-[4-2-Methoxyphenyl-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide maleate.

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Abstract

5-HT_{1A} receptors have been hypothesized to mediate some of the neuronal plasticity and behavioral responses stimulated by serotonin selective reuptake inhibitors. Although the cellular signaling pathways required for inducing these actions have not yet been determined, roles for the neuroprotective extracellular-regulated kinase (ERK) mitogen-activated protein (MAP) kinase and Akt pathways have been suggested. In the current studies we have utilized primary cultures to directly determine whether hippocampal 5-HT_{1A} receptors couple to activation of Akt and ERK. We found that E18 hippocampal neurons exhibit a twofold activation of Akt when exposed to nanomolar concentrations of 5-HT. The 5-HT_{1/7} receptor-selective agonist 5-carboxamidotryptamine maleate (5-CT) and the 5-HT_{1A/7} receptor-selective agonist 8-hydroxy-N,N-dipropyl-aminotetralin (8-OH-DPAT) maleate were found to activate Akt with equal efficacy, and similar potency, to 5-HT. p-MPPI and WAY-100635, antagonists selective for 5-HT_{1A} receptors, completely inhibited 5-CT-stimulated Akt activation. Activation of Akt was also inhibited by pretreatment with pertussis toxin as well as the phosphatidylinositol 3-kinase inhibitors, wortmannin and LY294002. In contrast, the 5-HT selective antagonist, SB269970, caused no inhibition. Although the density of 5-HT_{1A} receptors expressed by cultured neurons was sufficient to activate Akt, no activation of ERK was observed. These findings suggest that Akt, and not ERK, may be relevant to previous reports of hippocampal 5-HT_{1A} receptors mediating neurotrophic responses.

Medications that increase synaptic levels of serotonin (5-HT), such as the selective serotonin reuptake inhibitors (SSRIs), are effective treatments for depression and anxiety. While it is not known which of the at least 14 receptors for 5-HT mediate clinical response, an accumulation of data from both animal and clinical studies suggest a potentially important role for 5-HT_{1A} receptors. For example, a number of selective agonists have been shown to be similar to antidepressants in decreasing immobility in the forced-swim test (Wieland and Lucki 1990). Additionally, 5-HT_{1A} receptor knockouts do not exhibit SSRI-induced decreases in immobility in the tail suspension test (Mayorga et al. 2001), nor do they exhibit SSRI-induced decreases in latency to feed in the novelty-suppressed feeding test (Santarelli et al. 2003). Interestingly, the coupling of 5-HT_{1A} receptors to G proteins and to inhibition of adenylyl cyclase has been reported to be attenuated in suicide victims, suggesting a possible protective role for the receptor (Hsiung et al. 2003).

5-HT_{1A} receptors are expressed both as autoreceptors in the raphe and post-synaptically in such brain regions as the hippocampus. In the hippocampus, 5-HT_{1A} receptors are expressed at high density in regions CA1, CA3, and the dentate gyrus (Chalmers and Watson 1991). Stress and elevated levels of glucocorticoids have been shown to induce a number of deleterious changes in the hippocampus, including suppression of neurogenesis in the dentate gyrus (Gould et al. 1992, 1998). Conversely, antidepressants and 5-HT_{1A} receptor agonists have been found to stimulate hippocampal neurogenesis (Jacobs et al. 2000; Malberg et al. 2000; Santarelli et al. 2003). Moreover, receptor knockout mice do not exhibit SSRI-induced neurogenesis (Santarelli et al. 2003) and 5-HT_{1A} receptor antagonists decrease the basal rate of neurogenesis, as measured by bromodeoxyuridine (BrdU) labeling (Radley and Jacobs 2002).

The identities of the cellular pathways utilized by 5-HT receptors in the treatment of depression are currently unknown. However, it has been hypothesized that the pathways may be similar to those mediating antidepressant-induced neuroprotective changes in the hippocampus and other brain regions. Extracellular-regulated kinase (ERK) microtubule-associated protein (MAP) kinases and Akt (protein kinase B) are thought to be relevant, as they have been found

to confer neuroprotection in several models of apoptosis (Tamatani et al. 1998; Hetman et al. 1999; Matsuzaki et al. 1999; Yamaguchi et al. 2001). Although 5-HT_{1A} receptors have been found to couple to activation of ERK in a number of cell lines (Cowen et al. 1996; Garnovskaya et al. 1996; Mendez et al. 1999; Lin et al. 2002), it appears that this coupling may not occur in brain. Rats treated with 5-HT_{1A} receptor agonists have been reported to exhibit no activation of ERK in hippocampus, striatum, or frontal cortex (Chen et al. 2002). However, in vivo studies of cellular signaling in the hippocampus can be difficult to interpret. Systemic treatment with 5-HT_{1A} receptor agonists induces hormonal changes (Vicentic et al. 1998) that could possibly alter ERK activity. Additionally, acting through presynaptic autoreceptors, 5-HT_{1A} receptor agonists cause a reduction in synaptic 5-HT concentrations. These agonists would therefore be expected to inhibit changes in ERK activity mediated by all post-synaptic 5-HT receptors other than 5-HT_{1A} receptors. Such inhibition could mask 5-HT_{1A} receptor-mediated increases in ERK. Our present in vitro studies have therefore been designed to directly determine whether post-synaptic 5-HT_{1A} receptors couple to activation of Akt and ERK in hippocampal neurons.

Experimental procedures

Materials

5-HT, 8-hydroxy-N,N-dipropyl-2-aminotetralin hydrobromide (8-OH-DPAT), 4-Iodo-N-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-benzamide hydrochloride (p-MPPI), [R]-3-[2-(2-[4-Methyl-piperidin-1-yl]ethyl)pyrrolidine-1-sulfonyl]phenol (SB269970) and N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinyl-cyclohexanecarboxamide maleate (WAY-100635) were purchased from Sigma (St Louis, MO, USA). 5-Carboxamidotryptamine maleate (5-CT) was purchased from Sigma and Tocris (Ballwin, MO, USA). Recombinant human brain-derived neurotrophic factor (BDNF) was purchased from Alomone Laboratories (Jerusalem, Israel). Pertussis toxin, wortmannin, and LY294002 were purchased from Calbiochem (San Diego, CA, USA).

Cell culture

Hippocampal neuronal cultures were prepared as previously described (Errico et al. 2001; Lin et al. 2003). Hippocampi were isolated from embryonic day 18 (E18) Sprague-Dawley rats obtained from Hilltop Laboratory Animals (Scottsdale, PA, USA), and 10⁶ cells were plated per poly-(D-lysine)-coated 35-mm Petri dish. Cells were maintained in serum-free medium consisting of a 1:1 (v/v) mixture of Ham's F-12 and Eagle's minimal essential medium supplemented with 25 μ g/mL insulin, 100 μ g/mL transferrin, 60 μ M putrescine, 20 nM progesterone, 30 nM selenium, 6 mg/mL glucose, 7.5 units penicillin - 7.5 μ g streptomycin/mL at 37°C (95% air, 5% CO₂). Cells remained in culture for 5-7 days prior use. It should be noted that this is in contrast to our earlier 5-HT₇ receptor studies in which cells remained in culture 2 weeks (Errico et al. 2001; Lin et al. 2003).

A previously described transfected Chinese hamster ovary (CHO) cell line expressing human 5-HT_{1A} receptors at a density of 3000 fmol/mg membrane protein (Cowen et al. 1996; Mendez et al. 1999) was maintained in Ham's F-12 medium, supplemented with 10% dialyzed fetal bovine serum (dialyzed in membranes with 1000 Dalton molecular weight cut-offs against a 100-fold greater volume of 150 mM NaCl to remove endogenous 5-HT), 100 units penicillin - 100 μ g streptomycin/mL, and 400 μ g/mL geneticin at 37°C (95% air, 5% CO₂). The day prior to use, CHO cells were washed with phosphate-buffered saline (PBS) and cultured overnight under serum- and geneticin-free conditions.

Immunoblots

Monoclonal antiphospho-ERK1/ERK2 (Thr202/Tyr204), rabbit polyclonal antiphospho-Akt (Ser 473), and rabbit polyclonal antitotal Akt were obtained from Cell Signaling (Beverly, MA,

USA). Rabbit polyclonal antitotal ERK1/ERK2 and horseradish peroxidase-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Cells were treated with the addition of reagents directly to the culture media. Cells were then washed with PBS, and routinely lysed with a 26-gauge needle in 25 mM HEPES (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1 mM β -glycerol phosphate, 50 mM NaF, 5 mM EDTA, 1 mM sodium orthovanadate, 250 μ M 4-(2-aminoethyl)-benzene-sulfonylfluoride hydrochloride, 0.1% aprotinin, and 10 μ g/mL leupeptin. After 20 min on ice, the lysate was centrifuged at 10 000 g for 10 min at 4°C. Supernatant proteins were separated on 10% resolving gels (Bio-Rad Laboratories, Hercules, CA, USA) and transferred to 0.45 μ m Immobolin-P polyvinylidene difluoride membranes (Millipore Corporation, Bedford, MA, USA). Bound antibodies were visualized using Enhanced Luminol Chemiluminescence Reagent (Perkin-Elmer Life Sciences, Boston, MA, USA) and direct exposure to a Kodak Image Station 440CF with a cooled, full-frame-capture CCD camera (Kodak, Rochester, NY, USA). Net intensity of bands was calculated directly from stored images using Kodak Digital Science 1D Image Analysis Software (version 3.5) on defined regions of interest.

Statistical analyses

Results are expressed as the means $\pm$ SEM of three or more experiments, performed in duplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests.

Immunofluorescence

Cells were plated, as described above, in poly-(D-lysine)-coated 35-mm Petri dishes. Cultures were washed with PBS and fixed in methanol for 6 min at 20°C. Cultures were incubated for 30 min at room temperature (22°C) in PBS blocking buffer containing 10% goat serum (Vector Laboratories, Burlingame, CA, USA). Cultures were then incubated for 1 h, at room temperature (22°C), with a polyclonal antimicrotubule-associated protein (MAP)-2 antibody (1: 100), obtained from Santa Cruz Biotechnology, in the presence of 1.5% goat serum. Cultures were washed with PBS and incubated for 45 min with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (1: 200), obtained from Santa Cruz Biotechnology, in the presence of 1.5% goat serum. Cultures were then washed and coverslipped with Ultra Cruz Mounting Media (Santa Cruz Biotechnology containing 4,6-diamino-2-phenolindol dihydrochloride (DAPI). Stained cultures were viewed under fluorescence illumination.

Results

Activation of Akt by 5-HT and selective agonists for 5HT_{1A} receptors

We have previously used dissociated E18 neuronal cultures to demonstrate that 5-HT₇ receptors couple to activation of ERK in the hippocampus (Errico et al. 2001; Lin et al. 2003). No 5-HT_{1A} receptor-mediated activation of ERK was detected in those studies, in which neurons were cultured for 2 weeks prior to use. However, it was not clear that our culture conditions were optimal for studying 5-HT_{1A} receptor-mediated cell signaling. In contrast, our present studies utilized neurons cultured for 5-7 days prior to use. These were found to exhibit the largest magnitude of 5-HT_{1A} receptor agonist-stimulated Akt activation. It should be noted that our cultures are comprised almost entirely of neurons. Nearly all cells stain positively for the neuronal marker, MAP-2 (Fig. 1).

Treatment of cultured hippocampal neurons with 5-HT was found to stimulate an approximately twofold increase in the level of activated (phosphorylated) Akt (Fig. 2). Activation of Akt occurred at nanomolar concentrations of 5-HT, and was maximal at 100 nM. 5-carboxamidotryptamine maleate (5-CT), an agonist for 5-HT₁ and 5-HT₇ receptors, was also found to stimulate an almost twofold activation of Akt with a potency similar to that of

5-HT (Fig. 3a). Activation was maximal within 2 min of treatment with 5-CT. By 20 min, the level of activated Akt approached basal levels (Fig. 3b).

($\pm$)8-hydroxy-N,N-dipropyl-2-aminotetralin hydrobromide (8-OH-DPAT), an agonist selective for 5-HT_{1A} and 5-HT₇ receptors, was found to be similar to 5-HT and 5-CT (Fig. 4). A maximal, twofold increase in Akt activation occurred at 100 nM (Fig. 4). Significantly, activation was observed at nanomolar concentrations comparable to those seen with 5-HT and 5-CT. The similar potency of Akt activation by 5-HT, 5-CT, and 8-OH-DPAT was consistent with mediation by 5-HT_{1A} receptors (Raymond et al. 1992; Hoyer et al. 1994). In contrast, mediation by 5-HT₇ receptors would have been characterized by an EC₅₀ for 8-OH-DPAT higher than that for 5-HT and 5-CT (Ruat et al. 1993; Adham et al. 1998; Hemedah et al. 1999).

Activation of Akt is inhibited by 5-HT_{1A} receptor antagonists but not by 5-HT₇ receptor antagonists

The effect of p-MPPI and WAY-100635, antagonists selective for 5-HT_{1A} receptors, relative to 5-HT₇ receptors (Hirst et al. 1997; Ying and Rusak 1997) were tested. Both antagonists caused almost complete inhibition of 5-CT-stimulated Akt activation (Fig. 5a). In contrast, no inhibition of 5-CT-stimulated activity was observed when cells were treated with the 5-HT₇ receptor selective antagonist, SB269970.

5-HT_{1A} receptors do not couple to activation of ERK in hippocampal neurons

Though 5-CT was found to stimulate a twofold activation of Akt, no activation of ERK was detected (Fig. 5b). This absence of 5-HT_{1A} receptor-mediated ERK activation did not reflect a defect in neuronal signaling. Neurons treated with BDNF exhibited large, eightfold, increases in ERK activation in addition to activation of Akt (Fig. 6).

Activation of Akt is inhibited by pertussis toxin and PI3K inhibitors

5-HT_{1A} receptors most commonly couple to G_{i/o}-type G proteins (Gettys et al. 1994; Butkerait et al. 1995; Clawges et al. 1997; Lin et al. 2002). However, they have also been reported to possibly utilize G_z (Serres et al. 2000). Neurons were therefore pre-treated with pertussis toxin in order to determine whether the coupling of 5-HT_{1A} receptors to activation of Akt was mediated through G_{i/o}-type G proteins. Overnight pre-treatment of hippocampal cultures with relatively low concentrations of toxin (150 ng/mL) completely inhibited the activation of Akt stimulated by 5-CT (Fig. 7a).

Activation of Akt is most commonly mediated by a phosphatidylinositol 3-kinase (PI3K)-dependent pathway requiring 3-phosphoinositide-dependent kinases-1 and -2 (PDK-1 and -2; Alessi et al. 1996, 1997). However, PI3K-independent pathways have also been described (Filippa et al. 1999). Pre-treatment with 30 nM wortmannin and 25 μ M LY294002, two chemically different PI3K inhibitors, completely inhibited both basal and 5-CT-stimulated activation of Akt (Fig. 7b). Therefore, activation of Akt by 5-HT_{1A} receptor agonists proceeds through a pathway requiring G_{i/o}-type G proteins and PI3K.

A transfected cell line confirms that 5-HT_{1A} receptors couple to activation of Akt

We next used a previously characterized 5-HT_{1A} receptor-expressing transfected CHO cell line (Cowen et al. 1996; Mendez et al. 1999) to further demonstrate that we had identified a 5-HT_{1A} receptor-mediated pathway. 8-OH-DPAT was used as the agonist in these studies, rather than 5-CT, in order to avoid activating endogenous 5-HT_{1B} receptors. 8-OH-DPAT was found to stimulate a large increase in the level of activated Akt (Fig. 8). This increase was completely

inhibited by both pertussis toxin and WAY-100635. In contrast, neither pertussis toxin nor WAY-100635 reduced basal levels of activated Akt.

Discussion

5-HT_{1B} receptor agonists have been previously reported to activate Akt in transfected BE(2)-C neuroblastoma cells (Lione et al. 2000). 5-HT₂ receptors have been similarly found to couple to activation of Akt in cardiomyocytes, skeletal muscle cells, and PC12 cells (Hajduch et al. 1999; Nebigil et al. 2003; Johnson-Farley et al. 2004). However, the present studies are the first to demonstrate that 5-HT can activate the antiapoptotic pathway in primary neuronal cultures. Activation of the serine/threonine protein kinase, subsequent to phosphorylation at Thr308 by PDK-1 and at Ser473 by an unidentified PDK-2 (Alessi et al. 1996, 1997), has been shown to enhance neuronal survival (Hetman et al. 1999; Matsuzaki et al. 1999; Yamaguchi et al. 2001). This neuroprotection is thought to be mediated by inhibitory phosphorylation of a number of cellular substrates, including pro-apoptotic glycogen synthase kinase-3 (GSK-3), Bad, Caspase 9, and the Forkhead family transcription factor, FKHRL1 (Cross et al. 1995; Brunet et al. 1999; Datta et al. 1999).

Our finding that 5-HT_{1A} receptors do not effectively couple to activation of ERK in cultured hippocampal neurons is consistent with results from an in vivo study in which rats treated with 8-OH-DPAT did not exhibit increases in hippocampal ERK activity (Chen et al. 2002). In contrast, 5-HT_{1A} receptors have been found to couple to activation of ERK in a number of transfected cell lines (Garnovskaya et al. 1996; Mendez et al. 1999; Lin et al. 2002), including the CHO cell line used in this paper (Cowen et al. 1996). The reason for this difference is unclear. However, differences in receptor density and G protein population are possible explanations. We have previously found that the efficacy of 5-HT_{1A} receptor coupling to activation of ERK is dependent on receptor density - more so than the efficacy 5-HT_{1B} receptor coupling to the MAP kinase (Mendez et al. 1999). We have also found that 5-HT_{1A} receptors display a preference for coupling to ERK activation through specific members of the G_i family of G proteins. G_{ia1} and G_{ia2} were found to be more effectively utilized than G_{ia3} (Lin et al. 2002). It is therefore possible that the density of expressed receptor, or the population of expressed G_i-type G proteins, in the hippocampus is not favorable for coupling the receptor to ERK activation.

There is increasing evidence for 5-HT_{1A} receptor agonists having neurotrophic properties. For example, agonists have been reported to attenuate NMDA- and glutamate-induced apoptosis in mesencephalic cultures (Madhavan et al. 2003). Akt could be expected to be relevant to these neurotrophic actions. Our findings are also of interest within the context of the findings of an autopsy study by Hsiung et al. (2003). Brain samples from suicide victims were observed to have attenuated 5-HT_{1A} receptor/G protein coupling and additionally to have a lower level of basal Akt activity than did control samples. It was hypothesized that the decreased Akt activity might have been related to the attenuated 5-HT_{1A} receptor function. Our finding that 5-HT_{1A} receptors do, in fact, couple to activation of Akt suggests that this hypothesis is plausible.

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Fig. 1. Hippocampal cultures maintained under serum-free conditions cells are comprised predominately of neurons. Hippocampal cells, cultured for 5 days, were stained with anti-MAP-2. The nuclear dye DAPI was used to visualize all nuclei.

Fig. 2.

5-HT stimulates activation of Akt in cultured hippocampal neurons. Hippocampal neurons were incubated for 5 min with the indicated concentrations of 5-HT, and lysed. Supernatant was analyzed by immunoblotting with antibody to phospho-Akt (p-Akt). Membranes were then stripped and analyzed with antibody to total Akt. Results are expressed as the means $\pm$ SEM of five experiments, performed in duplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control. Representative immunoblots are shown.

Fig. 3.

5-CT stimulates activation of Akt in cultured hippocampal neurons. Hippocampal neurons were incubated for (a) 5 min with the indicated concentrations of 5-CT or (b) for the indicated period of time with 100 nM 5-CT, and lysed. Supernatant was analyzed by immunoblotting with antibody to phospho-Akt (p-Akt). Membranes were then stripped and analyzed with antibody to total Akt. Results are expressed as the means $\pm$ SEM of (a) five experiments or (b) six experiments, performed in duplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control. Representative immunoblots are shown.

Fig. 4.

8-OH-DPAT stimulates activation of Akt in cultured hippocampal neurons. Hippocampal neurons were incubated for 5 min with the indicated concentrations of 8-OH-DPAT, and lysed. Supernatant was analyzed by immunoblotting with antibody to phospho-Akt (p-Akt). Membranes were then stripped and analyzed with antibody to total Akt. Results are expressed as the means $\pm$ SEM of five experiments, performed in duplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control. Representative immunoblots are shown.

Fig. 5.

5-CT stimulates activation of Akt, but not ERK, and is blocked by selective 5-HT_{1A} receptor antagonists. Hippocampal neurons were incubated for 5 min with 100 nM 5-CT in the presence or absence of 1 μ M SB269970, 1 μ M p-MPPI, or 1 μ M WAY-100635, and lysed. Supernatant was analyzed by immunoblotting with antibody to (a) phospho-Akt (p-Akt) or (b) phospho-ERK (p-ERK). Membranes were then stripped and analyzed with antibody to (a) total Akt or (b) total ERK. Results are expressed as the means $\pm$ SEM of three experiments, performed in duplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests. *** $p < 0.001$ versus 5-CT. Representative immunoblots are shown.

Fig. 6.

BDNF stimulates activation of both Akt and ERK, demonstrating that neurons at day 5 of culture express the cellular components required for activation of ERK. Hippocampal neurons were incubated for 5 min with 100 nM 5-CT or 20 ng/mL BDNF, and lysed. Supernatant was analyzed by immunoblotting with antibody to (a) phospho-Akt (p-Akt) or (b) phospho-ERK (p-ERK). Membranes were then stripped and analyzed with antibody to (a) total Akt or (b) total ERK. Results are expressed as the means $\pm$ SEM of two experiments, performed in quadruplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests. * $p < 0.05$; *** $p < 0.001$ versus control. Representative immunoblots are shown.

Fig. 7.

Activation of Akt is inhibited by pertussis toxin and PI3K inhibitors. Hippocampal neurons were pre-treated (a) overnight with 150 ng/mL pertussis toxin (PTx) or (b) for 15 min with either vehicle (0.1% DMSO), 30 nM wortmannin (wort) or 25 μ M LY294002 (LY), and lysed. Supernatant was analyzed by immunoblotting with antibody to phospho-Akt (p-Akt). Membranes were then stripped and analyzed with antibody to total Akt. Results are expressed as the means $\pm$ SEM of (a) five experiments or (b) six experiments, performed in duplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests. (a) ** $p < 0.01$ versus control; (b) *** $p < 0.001$ versus 5-CT. Representative immunoblots are shown.

Fig. 8.

A transfected CHO cell line confirms that 5-HT_{1A} receptors couple to activation of Akt. CHO cells stably expressing human 5-HT_{1A} receptors were treated for 5 min with 100 nM 8-OH-DPAT (DPAT) in the presence or absence of 1 μ M WAY-100635, and lysed. Alternatively, cells were pre-treated overnight, where indicated, with 150 ng/mL pertussis toxin (PTx) prior to 5 min of treatment with 100 nM 8-OH-DPAT. Supernatant was analyzed by immunoblotting with antibody to phospho-Akt (p-Akt). Membranes were then stripped and analyzed with antibody to total Akt. Results are expressed as the means $\pm$ SEM of three experiments, performed in duplicate. Experimental groups were compared by ANOVA followed by Bonferroni post tests. *** $p < 0.001$ versus 8-OH-DPAT. Representative immunoblots are shown.